

ceeding those which produce the maximum elongation of iron. The greatest observed retraction of nickel is more than three times the maximum observed elongation of iron, and the limit has not yet been reached.

12. A nickel wire stretched by a weight undergoes retraction when magnetised.

III. "The Essential Nature of the Colouring of Phytophagous Larvæ (and their Pupæ); with an Account of some Experiments upon the Relation between the Colour of such Larvæ and that of their Food-plants." By EDWARD B. POULTON, M.A., of Jesus and Keble Colleges, Oxford. Communicated by Professor J. S. BURDON SANDERSON, F.R.S. Received April 11, 1885.

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1. *The Essential Nature of the Colouring of Phytophagous Larvæ.*

Phytophagous larvæ owe their colour and markings to two causes: (1) Pigments derived from their food-plants,* chlorophyll and xanthophyll, and probably others; (2) pigments proper to the larvæ, or larval tissues made use of because of some (merely incidental) aid which they lend to the colouring, *e.g.*, fat. A larva may be coloured by either or both of these groups of factors. It may be generally stated that all green colouration without exception, as far as I have investigated the subject, is due to chlorophyll; while nearly all yellows are due to xanthophyll. All other colours (including black and white) and some yellows, especially those with an orange tinge, are due to the second class of causes (as far as I am aware: it is, however, extremely probable that certain colours will be proved to arise from the modification of the derived pigments; and many observations make it probable that other colours may be derived from plants in the case of larvæ feeding upon the petals, &c.). The derived pigments often occur dissolved in the blood, or segregated in the subcuticular tissues (probably the hypodermis cells), or even in a chitinous layer, closely associated with the cuticle itself. This last situation has only as yet been proved in the case of the pupa of *Papilio Machaon*, but it is almost certainly true of many other pupæ. The colours proper to the larvæ occur in the hypodermis cells and in the cuticle. The commonest structural basis of variability or polymorphism in larvæ is afforded by the varying extent to which either of these factors takes part in producing colouration. Thus brown and green are by far the commonest instances of dimorphism, and when this is the case the former is due to larval pigment, the latter to derived plant pigments. It is very remarkable that there should be such an essential difference between the larvæ from the same batch of eggs, as far as the causes of colour are concerned. Mr. Raphael Meldola has very conclusively argued for chlorophyll as the cause of the colour in green larvæ in a paper in the "Proc. Zool. Soc." for

* It will be shown that the derived chlorophyll of larvæ has undergone chemical changes of which the effects appear in the altered spectrum, and in the great stability of the pigment when present in the animal blood or tissues. If, on the other hand, the chlorophyll be separated from the proteid with which it is united in the animal, it is so unstable that the colour disappears at once, and it is impossible to obtain it in solution by the use of the ordinary solvents of vegetal chlorophyll (or by any reagents of which I have made use). The spectrum and chemical characters are very constant, seeming to prove that the same definite changes are wrought by many species of insect. At the suggestion of Professor E. Ray Lankester, I propose to call this derived pigment *metachlorophyll*. The spectrum of the green blood of the pupa of *Ephyra Punctaria* differs from that of other larvæ and pupæ hitherto examined, and I propose for this form of derived pigment the term *Ephyra-chlorophyll*. There is not at present sufficient ground for giving a distinctive name to the derived xanthophyll.—May 4th, 1885.

1873, p. 155. He explains the relation between larval and vegetal colouration, by supposing that the larvæ have been rendered transparent by natural selection, and that thus the vegetal colours are themselves seen and give the colour to the larvæ. He points out that internal feeders are not green (their food being without chlorophyll), and he quotes Chautard ("Compt. rend.," January 13, 1873) as stating that he had discovered chlorophyll in the tissues of insects.

Mr. Meldola further expands this theory in his editorial notes to his translation of Weismann's "Studies in the Theory of Descent," Part II, "On the Origin of the Markings of Caterpillars," &c. Here he adduces the important evidence that "some few species of *Nepitula* (*N. Oxyacanthella*, *N. Viscerella*, &c.) are green, although they live in leaf-galleries where this colour can hardly be of use as a protection, but their food (hawthorn and elm) contains chlorophyll." He also quotes Chautard ("Ann. Chim. Phys." [5], iii, 1—56) in addition to the previous reference to the same author, stating on this authority that chlorophyll is a substance of great chemical stability, and that it resists the animal digestive processes. On referring to Chautard, I find that Mr. Meldola is mistaken in the statement that Chautard had discovered chlorophyll in the tissues of insects. In both papers referred to Chautard expressly states that the chlorophyll came from the digestive tract of the insects (*Cantharides*) experimented upon, and that he failed to obtain the bands after macerating the elytra in alcohol. He indeed states that chlorophyll resists the digestive processes, meaning that it is detected unchanged in the fæces. In a note to one of the papers ("Ann. Chim. Phys.") Chautard does not seem to be entirely satisfied with his proof of the absence of chlorophyll in the elytra of cantharides. He states that if present the chlorophyll must be much altered, and the colour of the elytra cannot be due to it, and he points out that the colouring matter is very insoluble in alcohol and ether. He alludes to Pocklington's researches (recently quoted by Dr. MacMunn), but only mentions and disputes the possibility of ascertaining the plant upon which the cantharides fed by a spectroscopic examination of an extract of these insects.

Dr. MacMunn in a paper on Animal Chromatology in the "Proceedings of the Birmingham Philosophical Society" (1882—3, vol. iii, Pt. II, p. 351), states that all previous attempts to find chlorophyll in the colouring of insects have led to negative results. (There is one partial exception to this in the case of Chautard's hesitation about chlorophyll in the elytra of cantharides, and there is also Pocklington's work on the same subject mentioned by Dr. MacMunn in a later paper.) He states that by concentrating light on the integument of the larva of *Pieris Rapæ* and examining with a micro-spectroscope, he found a band in the red which showed a resemblance to chlorophyll.

In the reports of the British Association at Southport (1883), Dr. MacMunn further states that on withdrawing the intestinal contents, the green colour and band disappear (Krukenberg also states that the band is due to the contents of the digestive tract, quoted in a letter to "Nature" by Dr. MacMunn for the week ending January 10th, 1885). I have not yet examined the larva of *P. Rapæ* from this point of view, but I think it is very probable that the green colour is due to the blood which may have been also lost when the contents of the intestine were removed. I shall show later on that chlorophyll (or some modification of it) passes through the digestive tract and tinges the blood of Lepidoptercus larvæ, and that the colour of green larvæ is often due to this cause alone. I am sure that the contents of the digestive tract cannot account for the ground colour of a larva (such as this), in which the green is at all bright and distinct. Furthermore, the blood is extremely likely to be lost in any manipulation of a larva, for it exists under pressure and exudes from a very small aperture. If the intestine was dissected out, there can be doubt of the loss of the blood.

Dr. MacMunn also alludes to the discovery of chlorophyll in the elytra of cantharides, and states that he has confirmed this observation. He believes that this chlorophyll is a synthetic production, and suggests that it may be used for protective purposes, but that probably a pigment of less complicated chemical constitution might answer as well. Of course this would not apply to the derived (and modified) chlorophyll of Phytophagous larvæ.

Dr. MacMunn's interesting discovery of entero-chlorophyll (which he believes to be synthetically formed) in the liver of many invertebrates, does not apply to the present question. (See "Proc. Roy. Soc.," vol. 35, No. 226, p. 370.)

2. *Methods by which the two Factors of Larval Colouration may be Recognised: Instances of their Occurrence.*

If the body of a small individual of the green form of a dimorphic (green and brown) *Noctua* larva be gently compressed, the green tint disappears from this part, and is replaced by faint yellow. The markings of the larva (apart from the ground colour) are unchanged. At the same time the tracheal system and other internal structures become easily visible. Conversely, the swollen uncompressed part of the body becomes of a far deeper green than it was before. This is especially well seen in the true legs, of which the colour is normally pale. Such effects are due to the fact that the green colour depends upon the green blood. (I use this word for the more correct term, hæmolymph.) This conclusion is confirmed by examining the blood itself, and by observing that the green ground colour of the larva dis-

appears on removing the bright green blood. If, on the other hand, a brown variety of the same larva be compressed, the results are much less marked, and chiefly dependent upon the difference in transparency of the compressed and swollen parts of the larva. The blood is generally found to be colourless or very faintly yellowish: hence the ground colour of the green form depends upon its blood, while that of the brown form depends upon its proper pigment. Intermediate colours are produced by intermediate conditions, and it is quite common to find a series connecting the green and brown larvæ. On compressing a greenish-brown larva, the green at once becomes apparent in those parts of the organism which are deficient in pigment, *i.e.*, the under surface and claspers; and indeed it may be generally stated that the colour due to the blood generally predominates in the lower part of the body, while that due to larval pigment usually predominates on the back. The most extreme brown varieties have often lost all colour in their blood (except when it is seen in a very thick layer). *Phlogophora Meticulosa* is a common species upon which these observations can be made. Not only are these two factors so variably developed in dimorphic or polymorphic larvæ, but the same variability is very commonly seen in a single life-history. Thus many green larvæ become brown after one of the ecdyses, or brown ones become green. Hence in the period preceding ecdysis the blood must have lost its colour (*P. Meticulosa* and many Noctuæ), or the larval pigment must have become so opaque as to obscure any more deeply placed colour (common among Geometers). There are often great advantages to be gained by a brown larva retaining the green colour in its blood. This is well seen in the case of *Ennomos Angularia*, of which the larva is an opaque Geometer, resembling the dark twigs of the elm. The pupal period is very short (in the middle of the summer), and the cocoon is consequently very slight. It is formed of a few leaves loosely bound together, between which the larva and afterwards the pupa, are easily seen. Here therefore the brown colour is inappropriate, and the whole of the larval pigment is discharged, allowing the green blood to give its colour to the larva during the quiescent period before pupation. The pupa is similarly coloured and is also green. (It is dimorphic, with two shades of green. These facts about *E. Angularia* have been communicated to the Entomological Society in a hitherto unpublished paper, but the explanation has not appeared before.) Furthermore, these larvæ, when very young, live upon the surface of the leaf they are eating, and at this time they are coloured green by their blood, the pigment having not yet been deposited in a sufficient amount to render the surface opaque. Hence the alternation of colours, which follows the corresponding succession of objects imitated, depends upon the shifting scenes resulting from the presence of green blood behind a superficial covering, which can be rendered

transparent and opaque alternately. Even in the most opaque Geometer larvæ, exactly resembling the dark twigs of their food-plants, the inner surfaces of the large posterior claspers are often green or yellowish. This is the one spot in the body which is always hidden, and here only is the pigment absent and the tint of the blood visible.

In some cases the green colour of the blood disappears, and is replaced by a tint which helps towards producing the brown colouration. Thus the larva of *Chærocampa Elpenor* is at first green, becoming brown at the beginning of the fifth stage (as a rule). Examining the blood of a pupa of this species, I found that it was of a reddish-brown colour, and must therefore have helped in causing the ground colour of the last stages. Although no traces of the green colour were to be seen, the spectra of modified chlorophyll and of xanthophyll were distinct in a considerable thickness of the fluid. Evidently the larva had not destroyed the pigments of the previous stages beyond the point at which they ceased to interfere with the effect of the later stage. The brownish tint in the blood of this brown larva plays a far less important part in the total colouration than the green colour of the blood in green larvæ, as is proved by the following considerations:—It is not uncommon to find green larvæ of *C. Elpenor* in the last stage, and it may be assumed that the blood has then retained the green tint. But even in these green larvæ there is a considerable amount of dark pigment in and near the four eye-like terrifying marks on the first and second abdominal segments, and the brown colour (where it occurs) is not obviously different from that of a normal brown variety. Again, in blown specimens of *Chærocampa Porcellus* the brown colour remains distinct and dark, although the blood has, of course, been removed. And yet, considering the very complete resemblances between the larvæ of *C. Elpenor* and *C. Porcellus* throughout their life-history, it is practically certain that the blood of the latter is similar in colour to that of the former.

I will now give instances of the segregation of the derived pigments in the subcuticular tissues (probably the hypodermis cells). This must be true of *Smerinthus Ocellatus*, inasmuch as this larva remains green after dissection when the blood has escaped. Again, the green ground colour of the larvæ of *Sphinx Ligustri*, *S. Ocellatus*, &c., turns brown on the dorsal surface before pupation, but is comparatively unchanged elsewhere, while the green blood is also unchanged in the pupa. This latter fact is also true of the pupa of *Smerinthus Tiliæ*, although the adult larva becomes purplish-brown without a trace of green remaining (even upon the paler under-surface). Hence these larvæ must be coloured by superficial segregation of the derived pigments probably strengthened by the colour of the

blood. On the other hand there are larvæ which entirely depend upon the green blood for their colour, and there is a transparency about such larvæ which at once distinguishes them from those above described. Thus green *Noctua* larvæ are usually of this kind. The species of *Noctua* which exhibits the most typically transparent green (that I know of) is the larva of *Gonoptera Libatrix* (although I have never investigated the constitution of the colouring in this species). Again, pressure has a comparatively slight effect upon the distribution of colour in larvæ of the former division, while it produces the greatest effect in those of the latter. I think it is very probable that in many cases the derived pigments may only exist in the blood in the early stages, and may be deposited later in the superficial tissues. Such an arrangement would entirely accord with a very common change in the appearance of green larvæ during growth. There is generally a marked difference in the colour of a green larva after its first meal, and before this the transparency is so complete that its tracheæ and other internal organs can usually be seen. But it does not therefore follow that the chlorophyll is at this time deposited in the superficial tissues (although such may be the case), for the facts may be accounted for by an increase in the amount of colour in the blood, and by a change in its tint (the probability of this will be shown presently).

3. *The Different Results of Methods of Preservation upon the two Factors in Larval Colouration.*

The derived pigment can always be distinguished from that proper to the organism, in larvæ which have been preserved in spirit, or as blown and dried specimens. After both processes the proper pigment is unchanged, being quite insoluble in spirit and uninjured by drying. The derived pigments, on the other hand, dissolve in the spirit from the blood and from the superficial tissues, while in blown larvæ the blood is removed (with the other contents of the body), and the chlorophyll in the superficial tissues rapidly fades, while the yellow due to the xanthophyll may persist for a long time. Looking over a dried collection, it is at once obvious that green larvæ, such as *P. Rapæ*, *S. Populi*, *S. Tiliæ*, *Notodonta Camelina*, &c., have entirely changed in colour, while others, such as *Liparis Auriflua*, *Bombyx Neustria*, *Odonestis Potatoria*, *Eriogaster Lanestris*, &c., remain perfectly life-like. Hairy, brightly-coloured larvæ are generally unchanged, as also are those distasteful forms which are protected by conspicuous colours (such as *Abraæas Grossulariata*, *Euchelia Jacobææ*, &c.). Smooth brown larvæ (such as the majority of Geometers and many Noctuas) have evidently lost some depth of colour, due to the removal of the contents of the body and the con-

sequent transparency; but the changes are slight compared with those shown by green larvæ, and are evidently due to the hypodermis pigment being in amount or in character insufficient to render the surface entirely opaque. In the instances mentioned above as being identical in appearance with the living larvæ the pigment is entirely opaque. In such larvæ some of the pigment is often deposited in the cuticle, and is therefore visible in the latter after being thrown off at ecdysis. As a rule the cast off cuticle is transparent and colourless, except the last one thrown off at pupation, which is generally discoloured and brown, because it is moistened by the fluid which covers the pupa, and which hardens and darkens into the protective layer of the latter. The effect in spirit specimens is even more striking than in the other method of preservation, for the green colour is not merely changed, but has entirely disappeared, leaving the surfaces colourless.

This is very obvious with larval colouration into which both factors prominently enter. Thus in *Dicranura Vinula* (kept for a year and a half in spirit) the green ground colour has entirely faded, and the spirit is yellow with dissolved xanthophyll, while the large purple area on the back with its white marginal line is entirely normal.

4. Colours due to Larval Tissues independently of Pigment.

There is one sentence in Dr. Weismann's essay (on the markings of caterpillars) which shows that he attributes importance to the deeply placed substances in producing larval markings. Speaking of the second stage of the larva of *Macroglossa Stellatarum* (p. 246, English translation), he says:—"A dark green dorsal line, which, however, does not arise from the deposition of pigment, as is generally the case, but from a division in the folds of the fatty tissue along this position." According to my experience the dorsal line is generally formed in this way when its colour is similar to that of the ground colour, except for a deeper tint. This division in the folds of fat is occupied by the dorsal vessel, and the latter can be distinctly seen contracting; its margins being thus shown to be coextensive with the dorsal line, which is therefore really due to the derived pigment in the blood. (This is true of *S. Ocellatus*, *Sphinx Ligustri*, &c.) But the superficial colours can be caused by an accumulation of internal tissues immediately beneath the transparent skin quite independently of pigment. Weismann implies in the above-quoted sentence that the fat exercises a modifying influence upon colour. But it may do more than this; it may be the only factor in certain markings. I have already described ("Trans. Ent. Soc.," April, 1884) a Phytophagous Hymenopterous larva *Nematus (Curtispina)* in which the dark-green dorsal line was due to the blood of the dorsal vessel, while on each side of this line there was

a broad white band. Examination proved that the white bands moved with the contractions of the dorsal vessel, and showed the characteristic structure of fatty tissue. Dissection and the use of the microscope confirmed these conclusions. The rest of the larva was green (due to its blood), with a slight black shading below the white bands. The shading was the only part of the colour due to larval pigment. The skin was so transparent that the tracheal system was quite distinct, and the fat was removed from all the conspicuous surfaces (except in the region of the bands) through which it would have been visible, and would have added other white markings. But this tissue was collected superficially along the median ventral line, producing a ventral white band, which was always hidden, for the larva rests with its ventral surface applied to the edge of a leaf.

Last autumn (1884) I found that fat can also serve as the vehicle for other colours. I was working at another of the *Phytophagous Hymenoptera*—the larva of *Cræsus Septentrionalis*, and I found that the yellow colour round the lips of the seven median ventral eversible glands is due to fat collected at these points, and the yellow colour of the posterior segments also depends upon the same cause, the colour being contained in the fat globules themselves.

It is extremely likely that fat will prove to be a more important factor in larval colouring than has been hitherto supposed.

5. *Changes in the Two Factors of Larval Colouration before Pupation.*

It has already been mentioned that the superficial derived pigments of *S. Ligustri* become brown in the dorsal region, before pupation, while the colour of the blood is unchanged. In *D. Vinula* the whole larva becomes reddish-brown, and in this case the green blood changes to brownish-yellow. The true larval pigment also changes before pupation, except when it is cuticular. Thus the larva of *E. Angularia* becomes transparent by the disappearance of dark pigment, and the green blood gives its colour to the larva. The green colour of the blood is generally retained in the pupal state, and it is often of great importance, as will be shown presently. The changes in colour before pupation alluded to above, are for the protection of the larvæ in their new surroundings, against which their old colouration would be very conspicuous. And this is therefore only true of a limited number of larvæ which wander in search of a place in which to pupate, or are exposed against a new background (as *E. Angularia*). In all larvæ, however (except those entirely coloured by cuticular pigment), the colours ultimately fade in the quiescent and contracted state immediately preceding pupation. Even in green *Noctua* larvæ coloured by their blood, the fat is no longer withdrawn from the surface, and the deep green tint can only be seen in the interstices of this lobulated tissue.

In some cases there are indications of quite new markings in the changes preceding pupation. Thus in the larva of *G. Libatrix* black patches appear on the sides of two (I believe) of the anterior segments, while green patches appear on corresponding parts of the larva of *Selenia Illunaria*.

6. *The Causes of Colour in Pupæ.*

In the pupæ of nearly all Heterocera (except the few that are protectively coloured like the larvæ, e.g., *Ephyridæ*, *E. Angularia*, &c.), the larval colours have entirely disappeared from the surface, and are replaced by the darkened opaque cuticle of the pupa. The derived pigments are, however, generally retained in the blood. Before the covering has darkened, immediately after the last ecdysis, the pupa possesses a colour partly due to its blood (when coloured), and partly to its fat body. The effect of the former is seen in the parts where the latter is absent, especially in the wings, legs, and antennæ. These parts are therefore green or yellow in the pupæ of which the blood is coloured by derived pigments. The rest of the body is white, and through the transparent cuticle the lobulated fat body can be distinctly seen. These appearances can only be witnessed for a very short time, since the semi-fluid surface rapidly darkens into the normal reddish-brown or black; although it is not uncommon for the colour of the blood to be seen through the cuticle of the wings—the most transparent part. There are, however, instances in which some of the true larval markings can be traced upon the pupa directly after emergence from the larval skin. Thus in *S. Ligustri* the oblique stripes are distinctly seen upon their respective segments. In a specimen placed in spirit before the appearances were obscured, the oblique stripes have darkened into brown, while the surrounding surface remains comparatively pale. This subject will repay future investigation, and I think it is probable that larval markings will prove to be commonly present upon the pupæ, if examined sufficiently early. When the pupæ of Heterocera are freely exposed, the constitution of their protective colouring is similar to that of the larvæ. Thus in the dimorphic (green or brown) species of *Ephyra* larva, the colour of the pupa follows that of the larva. The green is due to chlorophyll in the blood, and the colour is especially deep over the wings and adjacent surfaces. The depth of colour is produced in certain positions by reflection from a white surface of fat behind a layer of green blood, the light having thus traversed the fluid twice; while in other positions the pupa is transparent. There are also black markings in the *Ephyra* pupa, which are due to cuticular pigment, and therefore remain in the empty pupa case.

The pupa of *A. Grossulariata* is believed to be gaudily coloured

(black and yellow) as a signal of distastefulness, and the appearance is entirely due to cuticular pigment, and persists in the empty shell. The pupæ of *Rhopalocera* are nearly all exposed, and protectively coloured. Most of the colours are due to the above-described causes, derived or proper pigment; but in some cases they cannot be the result of pigment at all, and have not yet been sufficiently investigated (*e.g.*, the metallic colours of *Vanessa* pupæ). I have worked at the colour in the pupa of *P. Machaon*, which appears in two chief forms connected by intermediate varieties, the forms being coloured by derived and proper pigments respectively. Those coloured by derived pigments are yellow or green and yellow, the green being predominant in the anterior part of the body, especially the wings, &c., but also occurring in minute lines and masses mixed with the yellow. The other form is grey with dark markings. The blood is always bright yellow, with contained xanthophyll, but this does not aid the superficial colour, as the cuticle is opaque. The derived pigments are contained in a remarkable and very complicated layer beneath the true cuticle. This layer is very finely lamellated, but at the same time possesses a prismatic structure, being composed of columns arranged vertically to the surface, and themselves made up of parallel fibrillæ. It is cuticularised, and stains deeply in picric acid, but it is sharply marked off by a fine homogeneous membrane from the true cuticle above, and the subcuticular tissues below. The whole structure needs further investigation, but I believe that the layer is a cuticularised and very complex result of the differentiation of the hypodermis cells. The pigments are contained in the outer lamellæ, while the inner remain white. Although the layer is very firm and completely united to the true cuticle (which it resembles in consistence), neither itself nor its contents are beyond the reach of the physiological processes of the organism, for the pigments are withdrawn shortly before the emergence of the imago, and the wings of the latter are visible through the now transparent covering. At the same time the whole inner layer must itself disappear, for the empty pupa cases of this species are thin, and evidently composed of the true cuticle only, as in other pupæ; while the inner layer makes the whole pupal covering very thick, being itself many times thicker than the true cuticle. I have no doubt that this layer will be found commonly in *Rhopalocera*, and it is probably correlated with the extreme development of protective colours in the pupæ of this group. The pupal pigments upon the dark varieties, and in some parts of most of the green varieties, are placed in the true cuticle.

7. *The Ultimate Use of the Derived Pigments.*

In many species there is a great positive gain in retaining the derived pigments in the blood (or other part) of the pupa, for by their

means the ova are coloured green or yellow, and the pigments are passed down into the next generation. In this way the newly hatched larva is tinged, and the break in protective colouring is thus filled up, which would otherwise occur before the results of the first meal could become manifest. Hence it might be supposed that the pigments would be retained in the case of female pupæ laying green eggs, but not necessarily always in the case of males. Nevertheless the pigments appear to be retained equally in both sexes, the only partial exceptions (if any exist) being in the case of dimorphic larvæ (green and brown) of which the females are more generally green and the males brown, and I do not think that there are any authentic instances of such a division. In Newman's "Moths" it is stated of *C. Elpenor* "caterpillar green or brown, the males generally brown," but I have never heard of any confirmation of this statement, and furthermore, the existence of a brown female larva would have to be explained. Last autumn the only larva I could obtain of this species was brown, with brown blood in the pupa, and it proved to be a male. It may, however, be considered certain that the females are sometimes brown, for all collectors seem to admit that the brown variety is far commoner than the green. It would be well to investigate the subject from this point of view. But I have already stated that the derived pigments are present in the brown blood of this species—in fact the characteristic spectrum of green blood was faintly but distinctly recognisable. Thus it is possible that there is sufficient pigment to tinge the ova, if collected in a comparatively small compass. Another possibility is that the pigments may undergo a slight change rendering them colourless, and that they are reconstructed for the ova. Against this hypothesis it must be urged that some of the pigment certainly remains unchanged. With the exception of this unsettled question my observations point uniformly in the same direction. I have examined the blood in the pupæ of the following species which lay green eggs:—*Smerinthus Ocellatus*, *Populi*, and *Tilia*, *Sphinx Ligustri*. In all cases the blood is green. *E. Angularia* is also another instance, the larva before pupation, the pupa itself, and the eggs being greenish.

On the other hand, *D. Vinula* lays large eggs, but they are reddish-brown from a deposit in the chitinous covering, and resemble spots on the leaves—to the upper sides of which they are affixed—and the young larvæ are black and are protected in the same way. In this species the blood of the pupa has lost the green colour which it possessed in the larva, becoming brownish-yellow.

It is easy to extract xanthophyll in alcoholic solution from ova in the bodies of female moths which have been preserved as cabinet specimens for many years. The eggs, when tinged by derived pigments, seem to owe most of their colour to xanthophyll, and they are

generally of a very yellowish-green. In the case of *E. Angularia* the eggs present (on a microscopic examination) a yellowish ground colour, with lines and patches of green. The pigments, at any rate partially, are dissolved in oil globules. The ova are opaque, and are coloured by reflected light, although no doubt a certain depth of substance is penetrated. In the same manner the newly hatched larva is always yellower than one which has taken a meal, apparently due to the predominance of xanthophyll in the former. In some cases there seems to be a segregation of the small amount of chlorophyll to a special part. Thus the head of the newly hatched *S. Ligustri* is green, while the rest of the body is yellow. These facts of the predominance of xanthophyll in the ovum and young larva are no doubt to be explained by the great stability of this derived pigment. Due to the same cause, this substance can be extracted from dried and blown larvæ that have been kept for many years, while the chlorophyll has entirely disappeared. It is probable that the green or yellow colour of the ovum is protective, especially considering that large and therefore easily seen ova are most generally tinted in this way, and because they seem to be deposited on the side of the leaf against which they would be least conspicuous (as I have noticed in the case of *S. Ligustri*). But the chief use of the pigments must be for the young larva, which often rests for some time before feeding, which is active, and therefore more easily recognised, and which does not possess a firm resistant covering like that of the ovum.

8. *The Probable History of Larval Colouration.*

It seems probable that food in the digestive tract was the first cause of larval colour. In root-feeding and other colourless larvæ the dark contents of the digestive tract are distinctly seen through the transparent varieties, and in many transparent coloured larvæ the same cause certainly helps to confer depth of colour. It is also probable that a dark dorsal line, due to the fluid contents of the dorsal vessel, was a very early marking, as also must have been the external effect of superficially placed fat. The next step would probably be the passage of the plant pigments through the digestive tract into the blood, and next from the blood into the subcuticular tissues, finally into so remarkable a tissue as that shown in *P. Machaon*. The employment of true pigment seems to be on the whole posterior in date to the use of derived pigment, and the former at first appears on the highly protected surfaces, the back and upper parts of the sides, conferring a distinct advantage in its greater number of available tints. In opaque larvæ, such as many brown Geometers, the green tints are still seen on the inner surfaces of the large posterior claspers where it was unnecessary to replace the older method of colouration. But these

stages were by no means mutually exclusive, or even necessarily successive, for each new method was an additional resource, not often to replace the rest, but in nearly all cases to be used with them. Hence the one of the commonest associations is a ground tint partially due to derived pigments in the blood, and also sometimes segregated beneath the cuticle, while special markings are conferred by true pigments generally placed in the hypodermis cells, although sometimes cuticular in position. The derived pigments more often confer general resemblances, the true pigments, special resemblances. Again the association is well seen in the use made of fat as a reflecting surface behind the transparent green blood. The fact that the colour due to derived pigments is almost incidental at first is shown by the possession of green blood by certain leaf-mining genera entirely concealed from view, as is pointed out by Mr. Meldola.

We can now summarise the causes of colour in larvæ with some attempt at the historic order of their employment:—

- | | | |
|-------------------------------------|---|------------------------------|
| I. The internal tissues and organs | } | a. Digestive tract. |
| with ready made colour..... | | b. Fat. |
| | | c. Dorsal vessel. |
| II. The passage of derived pigments | } | a. The blood. |
| through the walls of the diges- | | b. The subcuticular tissues. |
| tive tract into | | |
| III. The appearance of true pigment | } | a. The hypodermis. |
| in | | b. The cuticle. |

The above causes will, I believe, explain all larval and pupal colours, except such cases as the metallic tints of certain pupæ which cannot be entirely due to coloured substances.

9. *Spectroscopic Examination of the Blood of Lepidopterous Larvæ and Pupæ.*

In the following investigation Zeiss's microspectroscope was always employed, and proved to be extremely delicate and convenient on all occasions. The instrument was very kindly lent to me by Professor Burdon Sanderson. A paraffin lamp was at first used as the means of illumination, and it acted very well for the less refrangible half of the spectrum, but in all later work bright sunlight was alone employed because of its immense superiority at the violet end.

The blood was always obtained in such a way as to prevent admixture with the contents of the digestive tract or any secretions. Success in this attempt is aided by the fact that the blood exists under considerable pressure, so that after a minute prick, only just penetrating the hypodermis, a considerable quantity at once issues. In larvæ the distal parts of the claspers were generally pricked, the spot being chosen because of its situation at some distance from the diges-

tive tract. If the digestive tract had accidentally broken, fragments of the food would have been found in the blood when examined under the microscope, whereas it was always perfectly clear, containing only colourless corpuscles, fat-cells, and minute spherules of fat, which gradually came to the surface. Pupæ were generally pricked through the cuticle of the wings, and here also a considerable quantity of blood emerged under pressure. The whole of the blood was obtained by pushing the abdominal segments inwards, and ultimately by gradually increasing compression of the pupa. Owing to histolytic changes, the weak and thin-walled digestive tract is broken, and a red fluid escapes, which is mixed with the last of the blood. By carefully watching for the first appearance of the red fluid, the blood may be obtained in a perfectly pure state, exactly resembling that of the larva in clearness and in microscopic contents. A considerable quantity of gas also emerges usually just before the appearance of the fluid contents of the digestive tract, from which the gas also probably comes. The blood is received into sections of glass tubes of various lengths, with the ends carefully ground. One end is cemented with Dammar varnish to a glass slide, and when the tube is filled with blood a cover-glass is placed upon the open end, and becomes fixed by the drying of the blood. In most cases the blood so prepared will keep for months without change. If, however, air be admitted, an opaque black clot is formed on the surface, and the rest of the blood becomes cloudy. It will also keep, I believe, indefinitely in sealed tubes.

The Spectrum of Unaltered Blood.—(a.) *Larvæ of P. Meticulosa.*—These larvæ were very suitable for investigation, as all varieties of colour between green and brown are common. The blood of a green larva was taken up (December 12th, 1884) by a capillary tube with an internal diameter of about $\cdot 75$ mm. The blood was allowed to stand four days, during which it evaporated to about half its bulk, but did not otherwise change; the tube was then sealed up. The colour was bright green, becoming darker after concentration. The spectrum was carefully noted (December 29th, 1884), a paraffin lamp being used. There was a broad band in the red, of which the extreme edges extended from $64\cdot 5$ — $68\cdot 5$, and when this band was best seen the violet end was cut off at 51, and the green was darkened to 52. There was no absorption of the red end. When the blood was fresh (December 12th, 1884) and less concentrated, the blue came through on the violet side of the darkening at 51, thus showing a broad dark band between this part and the green. A more concentrated sample of similar blood, prepared in the same way and at the same time, gave a darker band in the red with the same limits but with more defined edges. The violet end was similarly absorbed. There were indistinct traces of a broad dim band about 59 — $61\cdot 5$.

The two tubes superposed gave a more marked spectrum, but the need of stronger light was much felt. The dark band broadened, extending towards the red end to 69·5, and past 64 towards the violet, dimming the red, and apparently uniting with the dim band at 60. The edge of this latter was nevertheless slightly darker than the dimmed red. The absorption of the violet end was unchanged.

The fresh blood of a larva of the same species, which had been bright green (due to its blood), but which after ecdysis became brown, was examined (December 31st, 1884) in a wide capillary tube about 1 mm. in internal diameter. The blood was almost colourless, *very* faintly yellow, and produced no effect on the spectrum.

At the same time the fresh blood of another individual of the same species, which was dark greenish-brown, due to a combination of subcuticular pigment and green blood, was examined in a capillary tube.

The compound character of the larval colouring was proved by gentle pressure. The pale green blood with a thickness of about 1 mm. gave the band in the red from 65—68, the violet end, being completely absorbed at 45, darkened to 51 (when the slit was narrowed so as to render the band distinct). A greater thickness of blood darkened the band, and cut off the violet end at 50, darkening to 52 (when the band was distinct). A still greater thickness produced more marked results with nearly the same limits. On widening the slit no blue appeared at the absorbed end. The dark band now seemed to extend to 68·5. The whole spectrum was much dimmed, but this was probably due to the accidental presence of fat in the blood. In this case the thickness of the fluid was 3·3 mm., and the colour was bright green.

The fresh blood of another individual of the same species, which was of a mahogany-brown colour, due to subcuticular pigment, was examined at the same time. When the larva was gently compressed, it became slightly yellowish-green in the swollen uncompressed part. A thickness of 4·5 mm. was faintly yellowish.

The spectrum showed a *very* faint band in the red, and the violet end was cut off as before, only less sharply and completely. Thus the latter character is more delicate as a test than the former. Hence in this considerable thickness there was far less effect than that produced by the blood of the last individual in a capillary tube. After being exposed to the air for $2\frac{1}{2}$ hours the blood became brown, but there was no difference in the spectrum.

Another brown individual of the same species was examined at the same time. When gently compressed it emitted a clear green fluid from the mouth, exactly resembling the blood of a green individual in its colour and transparency when examined in a thickness of 2·25 mm. (except that this was a little darker).

The spectrum showed the band in the red from 65·5—68·5, while the blue was cut off at 50, darkened to 52. The band was very sharp and distinct.

The blood of this individual was also examined, and it was of the same colour as the last, and possessed the same spectroscopic characteristics. This observation upon the green fluid from this digestive tract is important, because it serves to identify the chlorophyll in the blood with that taken in as food. It is likely, however, that a greater thickness of fluid and the use of sunlight would bring out some differences between the derived pigment dissolved in the digestive secretions, and that united with a proteid in the blood. It is also noteworthy that the chlorophyll was present in solution.

All the observations hitherto recorded were made with the illumination of a paraffin lamp. Early in January I found how much more could be seen in these spectra at the violet end by the use of bright sunlight. About the middle of January the more concentrated of the two specimens of green blood in sealed tubes was examined by sunlight, and the spectrum mapped (see Chart, Spectrum 1). The band in the red, reaching from 64·5—68, was very black, except at the edges. When this band was most distinct and clear the violet end was absorbed to 51, darkened to 52. On opening the slit a little, the blue came through (though dimmed) at 48, the violet end being absorbed at 43. When the slit was very narrow, traces of another band, from 59·5—61·5, were faintly seen. The spectrum was unchanged at the end of March, although the blood had been exposed to light for a considerable time. The two chief bands and the absorption of the violet end were also seen in the blood of a living larva by passing the light through one of the claspers.

(b.) *The Pupa of Pygæra Bucephalus*.—The blood of this species is of a very bright and beautiful green colour. I have examined it on a great many occasions, with all conditions of daylight. In the Chart, Spectrum 3, is shown the spectrum of the thickness of 23·5 mm. examined with bright sunlight. The characteristic band in the red ends sharply at 71, gradually at about 64·5, passing into a lesser absorption of the red, which is continuous with the second band, extending from about 58—60·5, but with very indistinct limits. When these appearances are best seen the violet end is completely absorbed to 52, darkened to 52·5. On opening the slit a little, the dimmed blue comes through from 48—42. The band in the blue now sharply ends at 52, gradually at 48. Diminishing the thickness of the blood to 8 mm. produces nearly the same spectrum, the band in the red being a little narrower, while the band at D cannot be detected. On diminishing the thickness still further to 1 mm., another band appears in the violet. The spectrum is as follows :—The characteristic band from 65—70, the chief band

of the blue end 48—51, the second band of the blue end 45—46·75, the violet being absorbed at 41. The second band of the blue end is much fainter than the first band, and it is not seen in a thickness of 5 mm.

(c.) *The Pupa of S. Tiliæ*.—The blood was very bright green and perfectly clear. A thickness of 5 mm. was examined with the light of a paraffin lamp. The spectrum showed a very broad band in the red from 64·5—70, while the red and orange were much dimmed. The violet end was absorbed to 50, dimmed to 51, and no blue came through on widening. The green was the only part of the spectrum which was not dimmed. A thickness of about 1 mm. gave the band in the red from 66—69 (but the limits were very difficult to ascertain exactly), and the violet end was absorbed to 49, darkened to 51, while the blue came through on widening.

(d.) *The Pupa of S. Populi*.—The blood was examined by the light of a paraffin lamp, in a thickness of 5 mm. The colour was bright yellowish-green. The spectrum showed the broad band from 66—69, while the violet end was cut off at 51, darkened to 52. No blue came through on opening the slit (with this source of light).

(e.) *The Pupa of S. Ocellatus*.—The blood is of a bright yellowish-green colour (rather pale). I have examined many specimens by sunlight. A thickness of 4 mm. shows traces of the band in the red, and with great care and the right conditions of light, gives three bands in the blue end as well as an absorption of the extreme end. The chief band is from 48—50·3, the second from 46·75—45, and the third, which is very faint (and best seen when the slit is widened until the violet is absorbed at 41), occupies 43—42. With less thickness the characteristic band in the red disappears, while with greater thickness it becomes very distinct. After adding absolute alcohol to the blood, a bright yellow solution of xanthophyll was obtained, which gave the characteristic spectrum (shifted to the violet) 49—47, 45·25—44, the violet being absorbed at 42.

(f.) *The Pupa of S. Ligustri*.—I have also examined the blood of many individuals of this species by sunlight. The colour is a slightly greenish-yellow in individuals that have fed upon lilac in the larval state, yellow in those that have fed upon privet. The spectrum is marked by very slight effects at the red end, but very powerful absorption of the violet. In a thickness of 35 mm. the absorption in the red was strong, but did not approach that of a thickness of 23·5 mm. of the *P. Bucephalus*, although the absorption of violet was much more complete. The spectrum of this thickness of the blood of *S. Ligustri* is shown in the Chart, Spectrum 2, whilst Spectrum 3 is the *Bucephalus* blood which contrasts with it very markedly at both ends of the spectrum. The characteristic band is seen to extend from 70 (ending sharply) to 64·5, becoming gradually continuous with a less

absorption extending to D, and of which the part from 59—60 corresponds to the second band of the less refrangible part of the spectrum and the third band of true chlorophyll. The violet end is completely absorbed from 51·5, dimmed to 52, but on widening the slit a little blue comes through on the violet side of 48; but very dimly.

A thickness of 3 mm. gives no absorption of the red end, but shows three bands at the violet end and an absorption of the extreme end. The bands are (1), the chief band, from 50—48; (2), the second band, from 46·25—45; and (3), the third band, from 42—43; the violet being absorbed at 41. The bands become less distinct in the order above given, and between them the spectrum is dimmed. The second and third are best seen by an illumination from the bright sky near the sun rather than from the sun itself. This spectrum is shown in the Chart, Spectrum 6.

Comparing the spectra of the blood from pupæ of which the larvæ had fed upon different foods, it was found that the lilac-fed individuals showed greater effect at the red end than the privet-fed individuals, while the converse was true of the violet end. The comparison was made in a thickness of about 8 mm. and by sunlight.

(g.) *The Pupa of C. Elpenor*.—A single specimen of the blood of this pupa (from a brown larva) was examined (February 19th, 1885). The colour was a clear reddish-brown in thick layers, almost colourless in a thickness of 3·75 mm. It was examined in thicknesses of 23 mm. and 8 mm. In the former the spectra of derived chlorophyll and xanthophyll were distinct (although the colour was not perceptible). There was a faint band in the red from 69·5 (ending sharply), gradually lessening to 65. There was the chief band at the violet end from 50·5—48, and the second band was present from 46·25—45, and even the third band from 43—42, the violet end being absorbed at 41. These appearances were only seen in certain conditions of light. The first to disappear is the third band, for it fuses with the absorption at the end of the violet, which, therefore, seems to be absorbed to 43. This is common in other cases also. The lesser thickness also showed these effects at the blue end, but not at the red end.

(h.) *The Pupa of D. Vinula*.—The blood (of a single specimen) was of a reddish-yellow colour (by lamplight). It was examined in a thickness of 2·75 mm. by the light of a paraffin lamp (December 29th, 1885). There were no bands visible, but the violet end was absorbed up to about 50.

(i.) *The Pupa of P. Machaon*.—The pupæ are yellow, or yellow and green, or grey with dark markings. The blood of all varieties is bright yellow. The fresh blood in a thickness of 3·5 mm. gave the following spectrum in very good daylight. The chief band from 50—

48, very distinct; the second 46·25—45, less marked but distinct; the third 43—42, very indistinct; the violet end absorbed at 41. In all but the best light the violet end was absorbed to 43, and the third band ceased to exist separately. A thickness of 8 mm. tends further towards this absorption to 43, and the chief band extends to 50·5, or even a little further. The spectrum was the same in blood from all varieties. An alcoholic extract (90 per cent. spirit) of a grey variety was bright orange-yellow, and gave nearly the same spectrum as the blood, except that the bands were shifted to the violet end, and the third faint band was absent. The spectrum was: chief band 49—47, second 45·5—44, violet end absorbed to 42. The extract was examined in a thickness of 23 mm. and 9·5 mm.

A drop of water was placed upon a cover-glass and the latter was inverted upon the yellow back of a living pupa of *P. Machaon*, which was then examined by reflected light, being entirely opaque. The following spectrum was indistinctly seen:—A band in the red (very faint) 65—63, the chief band 49—47, the second 45·5—44, the violet end being absorbed to 42. The green parts of the same pupa, similarly treated, gave the following results:—69—64 in the red, 52—48. Pieces taken from the yellow parts of the pupal covering gave a similar spectrum to that above described when strong sunlight, concentrated by an Abbé's condenser, was passed through them; the green parts were more opaque, but the spectrum gave the chief bands distinctly. However treated, the spectra of the derived pigments still contained in the pupal covering are very indistinct. This is probably due to the nearly solid condition in which the pigments exist in association with some animal basis. The alcoholic extract of the covering, with its internal surface carefully washed to remove adherent blood, &c., gradually becomes yellow with dissolved xanthophyll. The green parts, carefully washed, dried, and placed in carbon bisulphide, gave a yellow solution with a slight greenish tinge, which gave this spectrum in a thickness of 24 mm. Band in the red 70—66, chief band in the blue 51·5—48, the violet being absorbed at 44. These appearances were indistinctly made out in bad light.

(j.) *The Pupa of Ephyra Punctaria*.—The pupa is green anteriorly, especially on the wings, and examined as an opaque object by means of Abbé's condenser (and a stop which blocked the central rays), with good sunlight, the following spectrum was seen:—Band in the red 70—65, a faint but distinct band 63—61, and the band 48—52, or 51 when the slit was widened; the violet absorbed at 43. This spectrum is remarkable as being the only instance yet observed in which the second band from the red end, of true chlorophyll, was seen separately, and occurred in the right order. For in all other pupæ and larvæ the third band appeared after the first, and *then* the second as a shading connecting the former two. The colour is entirely due to the

blood, which, however, does not after removal give a spectrum equal to that of the living pupa, because the amount (from one specimen) is so small, and is disposed in the pupa so as to produce a maximum effect.

(k.) *The Larva of S. Populi*, which had been blown and dried many years ago, was of a bright yellow colour (with a few small streaks and patches of green). The larval parietes were washed in water and placed in 90 per cent. spirit, which became bright yellow in a few hours, and gave the two bands of xanthophyll (shifted to the violet).*

(l.) *The Ova of E. Angularia* were of a yellowish-green colour. A single ovum examined as an opaque object gave the following spectrum: 51—48, 46·75—45, the violet being absorbed at 43. Although I could not detect the band in the red, I believe that a small proportion of chlorophyll exists in the ovum, as there are some bright green patches surrounded by the far more abundant yellow. The same spectrum was seen in the contents of the ovum which were also better treated by reflected light. The bands of xanthophyll were seen in the alcoholic extract of the crushed ova, the violet being absorbed to 43.

(m.) *The Ova of S. Tiliæ, Ocellatus, and Sphinx Ligustri* were taken from the bodies of moths which had been in my store-boxes for many years (8—10), and were well washed to remove any of the abdominal contents which adhered to their outer surfaces. The ova were then crushed in a mortar and extracted by 90 per cent. spirit, which in all cases yielded a yellow solution giving the spectrum of xanthophyll.

10. *Comparison of the above Results with those yielded by unaltered Plant Pigments*.—It is of importance for me to give the result of the spectroscopic examination of leaves by the use of the same instrument as that employed for the pigments in the larvæ and pupæ.

Two calceolaria leaves were superposed, gently compressed, and examined by sunlight, giving the spectrum shown on the Chart (Spectrum 4). There is a distinct black band from 70—64·5, two faint bands from 63 (nearly) to a little past 61, and from 60 (nearly) —57·5. There was also a dark band from 51—47·5, with the dimmed blue coming through between this and 43, from which the violet end was completely absorbed.

When five leaves were treated in the same way, results were obtained which are shown on the Chart, Spectrum 5, the three bands at the red end being wider, and the second and third much darker, while the violet end was completely absorbed up to 51, darkened to 52. The same results were seen in the leaves of other plants.

* After twelve hours the spectrum was as follows, in a thickness of 35 mm.:—chief band, 47—49, very distinct; second band, 44—45·5, faint but distinct; the violet end absorbed at 43.—May 4th, 1885.

Comparing these two spectra with those of green blood (not yellow) (Chart, Spectra 1, 2, and 3), the resemblance is seen to be very great, the chief differences being in the second and third bands of the red end, which are continuous (Chart, Spectra 2 and 3), while the third is developed before the second (Chart, Spectrum 1). Considering the chemical change which must have taken place in the chlorophyll during digestion, rendering possible the passage of the walls of the digestive tract, and considering its chemical union with a proteid constituent of the blood, the resemblances of the spectra are very striking; in fact, the two spectra are far nearer to each other than the ordinary spectrum of chlorophyll in alcoholic solution is to the unaltered chlorophyll of leaves. I believe, however, that the perfectly fresh alcoholic solution gives a spectrum resembling that of the leaf, but the spectrum changes in a few seconds, so rapidly is the solution acted on by light. I have never seen a band in the green in caterpillar's blood, and yet the blood is acid (Mr. Sorby states that any distinct band in the green is due to acidity).^{*} I have not been able to satisfy myself of the fluorescence of green caterpillar blood, certainly there is not the marked red fluorescence of the alcoholic solution of chlorophyll. But this red fluorescence is not present in leaves, nor in the perfectly fresh solution, according to J. Reinke ("Bied. Centr.," 1884, 692—696, abstracted in "Jour. of Chem. Soc." February, 1885, ii, 182). The points of difference between the derived xanthophyll spectrum of caterpillars (shown in Chart 6) and that figured by Mr. Sorby ("Proc. Roy. Soc.," vol. 21, No. 146, p. 442) is the occurrence of a third band in the former, the variable thickness of the second band and difference in darkness between it and the first, and the complete absorption of the violet end. It is possible that the latter may be due to one or more of the lichnoxanthines, while the third band is not always present, and is probably that to which Mr. Sorby alludes when he states (concerning the xanthophylls) that "sometimes three absorption-bands may be seen; but in that case it is generally easy to prove, either by chemical or photochemical methods, that the third band is due to a second substance." This third band was only seen in the best daylight with the greatest care, and it was never present in the alcoholic solution obtained from larvæ and pupæ, which gave the other two bands. It is rather a barren question to discuss whether more than one derived xanthophyll is present, until the botanists have agreed upon the classification of these substances

^{*} The spectrum of *Ephyra* blood is the same as that of the leaf, except in the absence of the third band; the slight difference in the chief band in the red being explained by difference in the amount of pigment examined. I think it is very probable that a sufficient thickness of this blood will give a spectrum identical with that of the leaf, but nevertheless the derived pigment must have been changed in such a way as to render it stable in solution.—May 4th, 1885.

(or even whether there are more than one. The immense complexity of the subject and the many shades of opinion are seen in a paper by Dr. A. Tschirch, "On the Preparation of Pure Chlorophyll," "Journ. of Chem. Soc.," February, 1884, p. 57).

Nevertheless, the difference between the spectrum of the yellow covering of *P. Machaon* and of its yellow blood, is just that figured by Mr. Sorby in his paper already quoted—a shifting of both the bands towards the violet end in the former case. At the same time it is probable that there is a difference in the animal substance with which the pigment is associated in these two cases, and this may account for some difference. The chlorophyll cannot have exercised a modifying effect on the results given above as due to xanthophyll (Chart, Spectrum 6), for when the spectrum of the latter was seen in the unaltered blood, the thickness employed was so slight that the band of chlorophyll in the red could not be seen, and further yellowish blood was made use of. In the blood of *P. Machaon* the bands of chlorophyll could not be seen even in a considerable thickness. When green blood (e.g., *P. Bucephalus*) was examined for xanthophyll, the latter was obtained in ethereal or alcoholic solution (the chlorophyll always disappearing with the latter solvent, becoming fixed in the solid state with the latter). On the other hand it is certain that the bands of xanthophyll are present in the spectrum of green blood (such as *P. Bucephalus*, Spectrum 3), the first band being contained in that from 52—48, and the second being rendered invisible because of the greater darkening of the violet between 48 and 42. The extreme absorption of the violet end shown in Spectrum 2 (*S. Ligustri*) is due to chlorophyll, xanthophyll, and, possibly, lichenoxanthine; and in this general absorption the chlorophyll takes a small part as compared with the next spectrum (Chart 3), as is at once seen on comparing their absorption at the red end.

11. *Conditions under which the Derived Pigments exist in the Larvæ, &c.*

There is one essential difference between the chlorophyll (especially) in solution in caterpillar blood or tissues, and all other solutions of the same substance, in the great stability of the former under the prolonged action of light. The necessity for this is obvious, inasmuch as the colour of the larvæ often depends upon their complete translucence (*P. Meticulosa*, &c.), and very many of them rest in such a position as to be freely exposed to direct sunlight. Furthermore there are long pauses in larval life, during which no food is taken to renew the derived pigments. Such occur at the various ecdyses, and the colours do not fade at these times, nor when the larvæ are from any cause deprived of food. Hence it is seen that the pigments, having

reached the blood or tissues, are in a state of great stability, and do not require renewal. The same thing is even more forcibly shown in the persistence of the pigments in the pupæ, and on into the ova and young larvæ of the next generation. But comparing these young larvæ before and after their first meal, and remembering that their latter appearance more resembles the one that they will permanently keep (if they remain green), it is obvious that something has been lost to the derived pigments in the interval between pupation and the hatching of the ova (often six months, or longer); and the constituent that has been partially lost is the more unstable of the two under all circumstances—the chlorophyll. It is not likely that the chlorophyll is partially eliminated by the action of light, for the pupal period is in nearly all cases passed in the dark. But it seems probable that pigment is lost in the various phases of redistribution gone through, as solution in fluid and deposition in tissue alternate. It is perfectly certain that the essential difference in colour between the newly-hatched larva (of a green species) and one that has fed, is the predominant yellow tinge and translucence of the former, and the green tinge and comparative opacity of the latter (not entirely due to the digestive tract being full).

The same unusual stability is also true of the xanthophyll, and must be due to the same cause, association with a proteid of the blood or tissue. The separation of the pigments from any constituent of the blood is at once effected by the addition of alcohol. If absolute alcohol be employed, the proteids are precipitated with the colouring matter, but the xanthophyll at once enters into solution in the alcohol, colouring it yellow, while the chlorophyll disappears, and the proteids are decolourised. This must be due to the extreme instability of the chlorophyll, at any rate in the presence of alcohol when separated from the proteid, so that it breaks up into colourless compounds when separated from it. The xanthophyll dissolved in the alcohol slowly decomposes in daylight, but lasts for many weeks, the colour gradually fading. Ether, on the other hand, precipitates the combined pigments and proteid in the form of a green jelly (the upper part is generally darkened), and for some hours dissolves no coloured constituent. Eventually, however, it becomes bright yellow with xanthophyll; while if the green coagulum be broken up in the ether more xanthophyll is dissolved, and a small quantity of green fluid containing chlorophyll remains at the bottom. This, however, is not dissolved in ether. The fragments of the coagulum undergo no apparent change.

If on the other hand the blood be protected from the air, the pigments seem to exist indefinitely, and the fluid does not decompose.* I

* In those instances in which the air has been entirely excluded (when a tube

have not yet tried exposing them to the prolonged action of bright sunlight, but they have frequently withstood the effect of its concentrated rays for several minutes during spectroscopic examination, and have been exposed for weeks to ordinary daylight.

But while the pigments exist unchanged in the blood of many larvæ for so long a time, in other species they are entirely destroyed during the comparatively short period preceding ecdysis, when some green larvæ become brown; and conversely the pigments may appear in the blood equally suddenly. The former change must be due to an active destruction or excretion of the pigments, and is probably also accompanied by changes in the digestive tract, whereby no more pigment is passed through its walls. And so also, as will be seen, the proportions of xanthophyll and chlorophyll may be changed during the life of a caterpillar.

The pigments present in the pupal covering of *P. Machaon* yield to solvent agents far more slowly than in any other place in which I have found them, but this may be due to the resistant nature of the tissue in which they are situated. It cannot be said that they are more stable than the pigments in the blood of the green *Ephyra* pupæ, for in both alike the pupæ are freely exposed to sunlight from autumn to early summer, without the possibility of the renewal of pigment.

It seems quite certain that the derived pigments of the blood and tissues are only protective, and play no further part in the physiology of these organisms. Thus there are no marked differences between the physiological processes of the brown and green individuals of the same brood in a dimorphic species, or in the processes of a green larva which has become brown, or *vice versâ*. It seems that the pigments are entirely harmless, and are often retained when they would have no effect upon colour. Thus in *P. Bucephalus*, the blood is bright green, although the larva and pupa are entirely opaque, while the eggs, I believe, are white. It is possible that in this case the conspicuous colours—which warn enemies that the species is distasteful—have

section has been completely filled and protected by a cover-glass) the blood has generally remained clear and undecomposed. When, on the other hand, the blood has been sealed up, a little air is unavoidably included, and the fluid generally decomposes, although the pigments are not always destroyed. The blood of the pupa of *S. Ligustri* was sealed up in a tube (with a little air) on March 20, 1885. On May 17 the colour and transparency were unchanged, but a whitish cloud had appeared on one side of the tube. On breaking off the end of the tube the contents were found to be putrid and strongly acid, and a large quantity of gas issued under considerable pressure. The whitish cloud was seen to be caused by the presence of large bacteria. The fluid did not clot or blacken on being exposed to the air, and the spectrum of the clear portion showed that the pigments existed unchanged. The spectrum was observed on May 22, five days after the contents of the tube had been exposed to the air and light, and two months after the blood had been taken from the living pupa.—May 22, 1885.

been recently acquired, and in consequence of the complete opacity, there would be no advantage in losing the colour of the blood.

It therefore appears that we must add a fourth class to the three spoken of by Dr. MacMunn ("Proc. Roy. Soc.," vol. 35, p. 387):—"In addition to the classes of chlorophyll-containing animals mentioned by Geddes, namely those which vegetate by their own intrinsic chlorophyll and those which vegetate 'by proxy,' so to speak, or by means of parasitic algæ, a third class must now be added: those which contain enterochlorophyll in their livers, or other appendages of the enteron." This fourth class comprises those animals which make use of a modified chlorophyll (and other plant pigments) derived from their food, because of the protective colour which they acquire from its presence in their blood or tissues.

12. *Notes upon the Physiology and Chemistry of the Blood of Larvæ and Pupæ.*

Some interesting points came out incidentally during the course of this investigation, and they are therefore mentioned, but only as a preliminary treatment of the subject which I hope to examine more completely during the present year. Much of this section is only suggestion.

Reaction.—The blood of all larvæ and pupæ examined (of all colours) was acid to litmus-paper, turning it distinctly reddish, with the exception of *E. Punctaria*, which seemed to be neutral (although only one specimen was examined). The acid which appears to be volatile can be extracted with ether, but I have not been able to obtain sufficient quantities of blood (during the winter) to determine what acid is present. The precautions previously detailed show how impossible it must have been to obtain any accidental mixture with digestive secretions. Besides, I have tested for the reaction of the blood upon a great many occasions, and again and again upon the same species. The corpuscles are well known to be amœboid, and Professor Schäfer tells me that as far as he is aware this is the only instance of their occurrence in an acid fluid.

Coagulation.—The blood clots after a very variable period of time, but generally darkens in about five minutes, ultimately forming a solid black clot which is due to oxidation. If blood be sealed in a tube, the small quantity of oxygen present will form a thin black film on the surface of the blood, and the action then ceases. I have already shown how blood can be kept indefinitely without clotting in a section of tube with a cover-glass over one end, and the other cemented to a glass slide. I have kept the blood of *P. Bucephalus* in this way for a month, quite unchanged, and on then breaking off part of the cover-glass a thick black crust was formed

on the surface, while the blood beneath became translucent instead of clear and transparent. On removing the crust a second thin one was formed, but on removing this no further coagulation took place. If in sealing up blood, or placing it in a tube section, a bubble of air is accidentally included, coagulation takes place round the bubble, but not elsewhere. This black substance is the normal clot, for the injured places on larvæ which have healed are always black, notably the horns of *Sphinx* larvæ which have been nibbled off by others of the same species. The coagulation takes place after the addition of water, or of a saturated solution of neutral salt (sodium sulphate). The occurrence of a reducing agent in the blood appears to be very remarkable, but it is possible that the substance is capable of again yielding up its oxygen, and so acting as a carrier. I have observed that if fresh blood be added to that which is turning black on the surface, the black clouds are redissolved. If this be not so, it is difficult to see how the blood can be the internal medium for the supply of oxygen in these animals, and one is tempted to the supposition that in the tracheal system we have a means for the supply of oxygen direct to the tissues. I hope to investigate the question this summer. Another suggestion which occurred to me was that the coagulation is a very similar process to the darkening of cuticular pigment on larvæ and the darkening of the pupal covering. It has always been assumed that this darkening is due to light, but it takes place rapidly and completely in pupæ buried several inches under ground, in compact and opaque cocoons, or sometimes in the heart of a tree. Furthermore, I have never observed that any attainable darkness made the least difference to the darkening of pupæ. I therefore think it very probable that this will also prove to be due to oxidation, and possibly to the formation of a substance similar to the black clot of the blood.

The brown and colourless blood darkens as well as the green, but the latter seemed to yield the most marked results, although I have not observed a sufficient number of instances, except in the case of the green blood, to be able to draw conclusions. There is great variability in the time that elapses before coagulation, and I have seen samples that have not clotted at all. From my memory of the blood of *S. Ocellatus* in the larval state, I believe that the blood coagulates far more rapidly than that of the pupa.

Action of reagents.—The effects of *ether* and *absolute alcohol* have been already described. The blood experimented upon was that of *P. Bucephalus*.

Fifty per cent. spirit, if poured carefully, lies on the top of the blood, and there are white clouds of precipitated proteids just above the junction. If shaken, the proteids and pigments are precipitated as yellowish-green clouds, and in a few minutes the upper part of

the liquid becomes blue, and ultimately black, from the formation of coagulum. The proteids are decolourised and sink, the alcohol remaining yellow with xanthophyll (the chlorophyll disappearing). *Absolute alcohol* does not lie on the top of the blood, but mixes with it at once.

Chloroform behaves in the same manner as ether, but it dissolves nothing coloured from the green coagulum; the latter contracts in a few hours, and a clear blue liquid appears between it and the sides of the tube. The exposed surface of the coagulum (the chloroform having sunk to the bottom) rapidly becomes black.

Distilled water, like weak spirit, lies on the top of the blood with a cloud of precipitated proteid (probably globulin) above the junction. On shaking, the cloud disappears, and the blood only seems diluted; if now more water be added (altogether many times the volume of the blood), in a few minutes the whole fluid becomes cloudy, remaining dark greenish. On filtering, a blue solution comes through, which slightly darkens for some hours. With less water the blood coagulates normally, although after a longer interval of time.

Carbon disulphide had no effect for a considerable time. Eventually the blood was coagulated (green) but nothing coloured was dissolved out.

Heat.—The blood of the pupa of *S. Ligustri* was heated in a glass tube in a water-bath; no change was seen till the temperature reached 132° F., when part of the blood became slightly dim. By 141° the whole of the blood was distinctly cloudy, but it was not till 180° that the blood became quite coagulated—solid-looking and opaque, the proteids being yellow with xanthophyll. In the interstices of the clot was a clear yellow fluid. The xanthophyll in the coagulum was easily extracted by ether or alcohol.

13. *The Relations between the Colour of Phytophagous Larvæ and that of their Food-plants.*

Entomologists have been long aware of the fact that the colours of many larvæ vary (within the limits of the same species) according to the colour of the plant upon which they are found. This is especially true of larvæ feeding upon brightly-coloured parts of the plant, such as the anthers or petals. At the same time there has been hardly any investigation of these interesting facts. The numerous instances in which such variations have been observed are, I believe, exhaustively recorded by Mr. Raphael Meldola in the editorial notes to his translation of Dr. Weismann's "Studies in the Theory of Descent" (the essay on the Origin of the Markings of Caterpillars), and in a paper in the "Proc. Zool. Soc." for 1873.

The first step in this investigation is to make quite certain of the facts by feeding a sufficient number of larvæ, from the egg, upon the

different foods which have been found to be associated with particular colour variations of the larvæ in field observation. Mr. Meldola mentions a paper by Mr. R. M'Lachlan ("Trans. Ent. Soc.," 1865, p. 453) entitled "Observations on some remarkable Varieties of *Sterrhæ Sacraræ*, Linn., with general notes on Variation in Lepidoptera." In this paper Mr. M'Lachlan gives some valuable data as to *Eupithecia Absynthiata*. The author collected about 100 larvæ of this species. When found upon *Senecio Jacobææ* they were yellowish, upon *Centaurea nigra* reddish, and upon *Matricaria* whitish. When nearly full grown they were all given *Senecio Jacobææ*, but this change of food did not affect the colour of the reddish and whitish varieties. From this Mr. M'Lachlan argued (1) that it was necessary for the larvæ to have fed on the one kind of flower from the egg to acquire the resemblance, and (2) that the colour is not caused by the food showing through the somewhat transparent integument. He thinks that variation may be indirectly caused by the food, and he points out the similarity of internal feeders. He also concludes that the indirect effect of the food in producing protective resemblance is normal, and the facts of opposite colouration must be due to other circumstances.

14. *Experiments upon the Larvæ of S. Ligustri.*

Mr. Meldola quotes (in the editorial notes) Mr. Argent for the observation that this larva is of a darker green when found upon *laurustinus* than when feeding upon lilac. He also quotes Mr. Davis for the opinion that the larvæ upon ash are of a more greyish-green than those upon lilac or privet. My own experience has always been that the larvæ are much duller in both the ground colour and the purple stripes when feeding upon lilac than when occurring upon privet. Those upon ash resemble the lilac forms. Mr. Davis' expression "greyish-green" exactly explains the difference between the lilac and privet forms (being applicable to the former). The privet varieties have a much brighter yellow ground colour, and the stripes are of a more vivid and redder purple, while the purple in the lilac forms is bluer and altogether duller (often tending towards brownish). Having often observed these differences in the field, I experimented upon some larvæ during the summer of 1884. The larvæ from a batch of eggs were divided into three lots and fed throughout their life upon lilac, privet, and ash respectively. The larvæ upon ash did not flourish, but about twelve of the privet larvæ and six of the lilac arrived at maturity, and without exception showed the differences I have indicated above. The results of these experiments have been communicated to the Entomological Society of London, and the two varieties figured, in a paper not yet published

15. *Experiments upon the Larvæ of S. Ocellatus.*

The variability of this species must have been observed by many entomologists. In Mr. Meldola's editorial notes there are the following references to this larva:—In 1879 Mr. E. Boscher found ten to twelve larvæ upon *Salix Viminalis* near Twickenham. These larvæ were of a bright yellowish-green ground colour, and all possessed the rows of red-brown spots which sometimes occur on this species. He also found eighteen to twenty larvæ of the same species feeding upon *S. Triandra*, in a locality not far distant, all of which were dull whitish-green above, passing into bluish-green below. None of these latter were spotted. In 1880 Mr. E. Boscher found seven larvæ on *S. Viminalis* in the same locality, similar to those found upon this plant the year before. He also found six larvæ upon another species of *Salix* in the same osier bed, two being of the bluish-green and four of the bright green variety.

It must be well known to all collectors that the bluish-green form occurs on ordinary apple trees in gardens. In 1880 Mr. Boscher conducted some breeding experiments upon this larva at Mr. Meldola's suggestion. The larvæ from three batches of eggs were fed upon *S. Triandra*, *S. Viminalis*, and apple respectively. Only three of the third batch survived, and were all of the bluish-green form.

I have added the following evidence to the case of *S. Ocellatus* ("Trans. Ent. Soc. of London," Part I, April, 1884). *Salix Rubra* and *S. Cinerea* produce the bright green variety, and crab also (as far as I could remember). On the other hand my experience had been that *S. Viminalis* produces whitish-green varieties. Figures of the two varieties are given in my paper. It must not be supposed that the yellowish-green varieties are always spotted, or even commonly so, but I believe that an instance of a spotted bluish-green variety has not been hitherto recorded.

My own experiments upon this species were conducted during the summer of 1884. The eggs were obtained from Mr. W. Davis, of Dartford. The larvæ after hatching were divided into five lots of six each, and they were fed upon the following food-plants:—apple, crab, *Salix Viminalis*, *S. Cinerea*, *S. Rubra*. As I was travelling at the time, it was sometimes difficult to obtain the right plants. On such occasions I made use of a plant which I believed (from an examination of its leaves) would produce the same colour as the one I had started with. The only instances of this were—once or twice in the case of crab, when I substituted an apple with similar leaves (green and glabrous underneath), and in the case of *S. Rubra*, for which *S. Babylonica* was given once or twice and *S. Triandra* once. I had previously ascertained (in the summer of 1883) that no effect is produced by feeding a larva for several days upon a food-plant which

tends to produce an opposite colouration. Hence the results of the experiments are as convincing as if I had been able to use the same plant throughout (as I did in three cases out of the five).

The larvæ hatched July 15th—18th, and I thought that there was some difference in colour due to the food, as early as July 27th (in larvæ hatched July 18th and fed on *S. Viminalis*), when the larvæ were about 15 mm. long, and advanced in the second stage. On careful comparison, the backs of those feeding on *S. Viminalis*, apple, and crab appeared very slightly whiter green than those feeding on *S. Cinerea* and *Rubra*. Before this all the larvæ were yellowish-green, much resembling the brighter variety. These early differences were *very slight*, and remained so for a long time. The head and two or three anterior segments were yellow-green in all the larvæ up to the end of the third stage, but this colour extended backwards for a less distance and was less marked in the whiter varieties. While there seemed to be some differences, all the larvæ were of a much whiter green than at first. In the third stage there was a blue tinge (especially seen in those feeding on apple) about the whitish-green on the backs of the whiter varieties, which became distinct on placing them beside the others. The white bluish-green appears on the back just above the subdorsal, and this line forms the boundary between the lighter area above and the darker surface below. Sometimes a line along this margin, even lighter than the colour of the back, indicates the position of the old subdorsal.

There was not this sudden transition between the upper and lower surfaces in the *S. Rubra* and *S. Cinerea* larvæ, although the subdorsal was sometimes recognisable. The larvæ feeding on crab were of the lighter variety, contrary to my expectations. As the larvæ advanced in the third stage, the differences increased and became quite distinct, but they did not approach those observed upon adult larvæ in the field. In the fourth stage the differences still continued to increase, although but slightly. The heads of the whiter varieties were still yellowish. It was very noticeable now, as at all times, that the apple, crab, and *S. Viminalis* larvæ were much whiter and nearer the well-known variety than the *S. Rubra* and *Cinerea* larvæ were yellow-green, like the other variety. Furthermore, the *S. Rubra* larvæ were rather yellower than those feeding on *S. Cinerea*. Towards the end of the fourth stage the whiter larvæ (except those on *S. Viminalis*) had become as white as those adult ones commonly found upon apple, and exactly resembled them in colour. On the other hand, the *S. Rubra* and especially the *S. Cinerea* larvæ were far from resembling the yellowish-green variety usually found on these plants, although very different from the larvæ upon apple, &c. The same facts were true of the last stage (fifth). The *S. Rubra* larvæ were the yellowest green, and as the effect of the apple, &c., had been so much stronger

than the *S. Rubra*, &c., the food of the yellowest variety was changed, and it was fed upon apple from the beginning of the fifth stage (August 14th. The larvæ were hatched July 17—18).

By August 23rd most of the larvæ were full-fed, and a careful comparison was made, with the following results:—

Apple.—The five adult larvæ are quite typical whitish-green forms.

Crab.—Only one of the five larvæ is nearly adult, the others far behind. The larvæ were hatched a day earlier than any of the others (July 15), and yet they have been backward all through. This is probably an effect of the food. The larvæ are almost as white as the others (becoming quite so afterwards).

S. Viminalis.—The four adult larvæ are not so white as the apple or crab, but are almost intermediate forms.

S. Cinerea.—The four larvæ may be called intermediate, and they much resemble the *S. Viminalis* forms, but are as yet younger and rather smaller (the tendencies towards the two varieties slightly increasing with growth).

S. Rubra.—The four adult larvæ are far more different from the apple varieties than any of the others, but they are not greatly beyond intermediate forms. The one fed upon apple for nine days was the brightest of all; it is now rather whiter than the others, which were still fed on *S. Rubra*, although it does not approach the apple or crab larvæ. But any change at all (as proved by the experiments to be described) in so short a time shows the very strong tendency of the whole of these larvæ towards the white variety. On August 26th this larva was adult without further recognisable change in colour.

This then was the result of the experiments. No one who compared the *S. Rubra* with the apple larvæ could hesitate for a moment in coming to the conclusion that the ground colour is largely affected by the food-plant. But at the same time there was no doubt that this is not the whole explanation of the differences observed in larvæ in the field, for my *S. Rubra* and *S. Cinerea* larvæ were not yellow-green varieties, but only intermediate. A strong tendency was manifested in the larvæ all through towards the white variety, and the food-plant could only overcome this to the extent of producing an intermediate form.

16. *Observation in the Field upon Larvæ of S. Ocellatus during 1884.*

I will now describe the results of my observations in the field during the past summer, which throw great light upon this question. The larvæ of *S. Ocellatus* were abundant last year, and I will give a list of all found to indicate the proportion of forms which were of exceptional colour (in relation to their food-plants).

August 4th.—Upon *S. Viminalis* on the River Cherwell, near Oxford, an almost full-grown larva of the whitish-green variety (normal according to observation).

(About) *August 7th.*—Upon (probably) *S. Ferruginea** (Anderson), on the Cherwell, two almost full-grown larvæ of *nearly opposite varieties and both on the same tree*. One was a bright yellowish-green variety (I believe abnormal on this tree), the other inclining towards the whitish-green side of an intermediate form (probably representing the tendency of this food-plant, and therefore normal).

(About) *August 7th.*—On *S. Rubra* (Cherwell) three larvæ; two just entered the last stage, one quite small in the third stage. All yellowish-green (normal), and one of the larger ones with brownish-red spots round all the spiracles except the first.

August 11th.—On *S. Ferruginea* (Cherwell: the same tree as that on which the two opposite forms were found, as described above); two larvæ at the close of the fourth stage, and both inclining to the white side of an intermediate variety (probably normal).

Also on *S. Viminalis*, a full-grown bright yellowish-green larva (abnormal according to my observations). The larva may have wandered from a neighbouring *S. Rubra*, but this is not likely, as the *S. Viminalis* was much eaten and the other apparently not eaten at all. On other occasions I have found the interlacing branches of *S. Viminalis* and *S. Rubra* eaten in such a way as to suggest that a larva had wandered from one to the other.

August 14th.—Upon *S. Smithiana*, at Botley, near Oxford, a full-grown larva inclining to the yellow side of an intermediate variety (probably slightly abnormal). Also upon *S. Smithiana* at Boar's Hill, near Oxford, a full-grown larva inclining to the white side of intermediate variety (probably normal). Also upon *Pyrus Malus* (var. *Acerba*) at Boar's Hill, an adult, very bright yellowish-green larva (probably normal). In this last case there were the brownish-red patches in front of and behind the eight posterior spiracles, but none of the other series of spots which are sometimes present.

August 16th.—Upon *S. Rubra* (Cherwell) an adult intermediate variety (abnormal).

August 17th.—Upon a small tree of *S. Babylonica*, in a garden at Oxford, six larvæ, of which five had just entered the fifth stage, or were passing through the last ecdysis. These were intermediate

* The tree in question (of which I have only seen a single specimen) was referred in the autumn of 1884 by Rev. J. E. Leefe and Mr. G. C. Druce to this species, while Mr. Bennet suggested *S. Rugosa*. This spring (1885) I have submitted the catkins to Mr. G. C. Druce, who has kindly sent specimens to other authorities, with the following results: Mr. S. G. Baker, of Kew, states that it is typical *Smithiana*, while the Rev. J. E. Leefe and Mr. Druce adhere to their former opinion that the plant is *S. Ferruginea*.—May 22, 1885.

varieties with (at present) more or less tendency towards the white-green variety, but on the whole very uniform (probably abnormal). One larva was rather more advanced and well in the fifth stage. It was a bright yellowish-green variety (probably normal).

August 18th.—Upon the same *S. Babylonica* one more intermediate variety, similar in size and colour to the five found on the 17th (probably abnormal).

September 2nd.—Upon an apple in a garden at Reading, a nearly adult bright yellowish-green variety (rather whitish on the back, but not nearly so much so as an intermediate variety). The apple leaves were white underneath, and in all previous experience such a tree has produced the most extreme form of whitish-green larvæ. Hence the occurrence is highly abnormal. In addition to the above, two larvæ were sent to me—one whitish-green variety, found upon the ground of another garden in Reading, and doubtless feeding on apple, and normal: another from the same garden as that in which I found the larva on September 2nd. This larva had turned brown, and was found upon a gravel path, but it was described as bright yellowish-green, and it bore traces of having had this colour when it reached me. It was found in the part of the garden where the apples grow. In quite another part at a considerable distance there is a tree of *S. Babylonica*, but it is not at all probable that the larva fed upon this plant. On the whole there is strong evidence for believing that this larva was another bright yellowish-green variety feeding abnormally upon apple.

17. *Experiments upon Captured Larvæ of S. Ocellatus.*

Experiments were made upon some of these larvæ to ascertain if it was possible, by changing the food, to modify their colours after a definite tendency had appeared. Such experiments could of course only be applied, with any hope of success, to the youngest of the above-mentioned larvæ.

The bright yellowish-green variety in the fifth stage (with brownish-red patches) found August 7th, on *S. Rubra*, was given apple August 8th, its length (when extended) being 40 mm. On August 14th, this larva was 58 mm. long when measured at rest in the sphinx attitude. It was now full-fed and became brownish. The colour was quite unchanged up to this point, and thus an immense amount of food can be eaten and great additional weight gained without any effect on the colour.

The other very similar larva found on *S. Rubra*, August 7th, was not quite so yellow as that just mentioned, but by August 10th it had become about the same. It was now fed upon apple, and was adult (and turned brownish) August 18th, without any recognisable dif-

ference in consequence of the change of food. The small larva (in the third stage) found upon the same plant, on the same date, was also fed at once upon apple. At this early stage the larva was as bright a specimen of the yellowish-green variety as I have ever seen. The apple made no difference in its colour, although it was eaten for many days. The larva had been slightly injured, and died without entering another stage. Evidently it possessed an even stronger tendency towards the yellowish-green variety than my bred specimens showed towards the other form, since the colours were very marked, while the larva was so small.

The two whitish larvæ in the fourth stage, found on August 11th, on *Salix Ferruginea*, were fed upon *S. Triandra* or *S. Rubra* during the whole of the fifth stage. On August 24th these larvæ were adult (and one of them browned): some slight effect had been produced, for they were both of them less white than was to be expected from their appearance when found.

The seven larvæ found August 17th and 18th upon *S. Babylonica*, were fed upon the same food-plant, and carefully compared. The bright yellowish one, which was more advanced than the others, became adult (and turned brownish) August 25th. It remained much the brightest all through. The others became adult a few days later, and throughout remained intermediate forms with a variable tendency towards the yellowish variety.

The tendency of the food-plant was seen during the growth of these six larvæ in the fifth stage, for they were whitish intermediate varieties at the beginning of this stage, and they inclined the other way at its close. The other larvæ were too old for experiment.

18. *The Protective Character of the Variation in the Larvæ of S. Ocellatus.*

These larvæ are protected by resembling the colour, and, to some extent, the veining of the underside of the leaves of their food-plants. The relations between the ground colour of the larvæ and the undersides of the leaves are shown in the following table, as far as these data have been indicated by any observation or experiment hitherto recorded:—

Food-plant.	Effect on <i>S. Ocellatus</i> .	Colour of undersides of leaves.
Apple	Strongly tends towards whitish varieties according to all observers, and also proved by two sets of experiments (Mr. E. Boscher's, quoted by Mr. Meldola, and my own). Exceptions very rare, and only known in 1884.	White undersides : pubescent.

Food-plant.	Effect on <i>S. Ocellatus</i> .	Colour of undersides of leaves.
Crab (cultivated)	Probably variable. I have found yellowish varieties on "yellow Siberian crab." My experiments showed that larvæ with a tendency towards whitish were not modified.	Undersides vary from green to white, but are smoother than apple, and sometimes glabrous.
Crab (wild) . . . Var. <i>Acerba</i> .	Probably strongly towards yellowish, but only one observation (which supported this view).	Undersides distinctly green and glabrous.
<i>S. Viminalis</i> . . .	Apparently contradictory results. From a fair number of observations in the field (with only one exception) I have assumed that the effect is towards the whitish variety. Mr. Meldola's instances, which are about 18 in number) oppose this conclusion. My breeding experiments perhaps supported Mr. Meldola's instances rather than my own, but they were not very conclusive. My experiments showed that this plant does not produce so white a green as apple, and this I think is the case. But it certainly does not cause so yellow a green as <i>S. rubra</i> . I am still inclined to think that it may tend towards whitish, but the experiments must be repeated.	Undersides white, with dense satiny down.
<i>S. Smithiana</i> . . .	Two instances, one inclining towards the yellow side of intermediate, one towards the whitish side. The latter, I think, represents the real tendency.	Undersides white, with dense satiny down.
<i>S. Ferruginea</i> ,* <i>Anderson</i> (probably)	Four instances upon a single tree, doubtfully referred to this species by Rev. J. E. Leefe. The leaves were exactly like those of <i>S. Smithiana</i> , only smaller. Three of the larvæ inclined towards the whitish side of intermediate, while one was bright yellowish-green. The former is doubtless the real tendency, and if so, indirectly supports my impression as to the tendency of <i>S. Viminalis</i> .	Undersides white and rather silky.
<i>S. Cinerea</i>	Only one instance, the yellowish variety, upon a tree of which the leaves had dark or glaucous undersides, and were not downy (at any rate distinctly). This probably represents the tendency of that particular tree, but in many cases it is	Undersides reddish, glaucous, or ashy, downy sometimes, and varying in the amount of down when present. A very variable species.

* See note upon this species in paragraph 16.

Food-plant.	Effect on <i>S. Ocellatus</i> .	Colour of undersides of leaves.
	likely that the opposite effect may be produced when the leaves are downy and white underneath. My experiments produced intermediate forms, but they are not very conclusive, for the larvæ could not be fed upon the same variety of <i>S. Cinerea</i> , as I was travelling about at the time. On some occasions I was obliged to use leaves with white pubescent undersides, which I believe tend towards whitish-green.	
<i>S. Triandra</i> ...	Mr. Meldola's instances (about 19) point towards the tendency being in the direction of whitish-green. My own experiments upon the modifying effect produced upon the whitish larvæ by feeding on this plant throughout the fifth stage, rather tend towards the other direction. From the colour and surface of the leaves, I should certainly have thought that the tendency is strongly towards yellowish.	Undersides green and glabrous.
<i>S. Babylonica</i> ...	Of seven instances, one was yellow and six intermediate, inclining towards the yellowish side. The former I believe represents the tendency of these leaves, which much resemble those of <i>S. Rubra</i> .	Undersides green and glabrous.
<i>S. Rubra</i>	A fair number of instances in favour of a very strong yellowish tendency. Only one exception (intermediate). Experiments confirm the observations, as yellowish intermediate forms were produced from larvæ, strongly tending towards the whitish variety.	Undersides green and glabrous.

Looking at the above list, I am strongly convinced that the tendencies of one of the food-plants of this larva can be ascertained by looking at its leaves, and that leaves with white pubescent undersides, as a rule, produce whitish varieties, while those with green glabrous undersides generally produce yellowish forms.

The greatest support to the theory that the colour and texture of the undersides of leaves is the best criterion of the effects is shown by the very strong cases of apple and *S. Rubra*. The same theory is supported by crab, *S. Cinerea*, and *S. Ferruginea*, as far as their evidence goes, and *S. Smithiana* is certainly not in opposition to it. *S.*

Babylonica is also strong evidence, if we assume (as there is some reason for doing) that the six intermediate larvæ had tended towards the whitish variety. *S. Triandra* and *S. Viminalis* remain a difficulty, but there is some confliction of testimony. The cases of *S. Smithiana* and *Ferruginea* are favourable to my view of the effects of *S. Viminalis* and of *S. Triandra*, although indirectly, for the leaves of the former species are intermediate between those of *S. Caprea* and *S. Viminalis* (*S. Smithiana* may be a hybrid between these two, and *S. Ferruginea* a variety of *S. Smithiana* or a hybrid between it and *S. Cinerea*. The leaves of the *S. Ferruginea* spoken of exactly resembled those of *S. Smithiana*, except that they were smaller.) Hence if *S. Viminalis* tends towards yellowish, it would be expected that *S. Ferruginea* would tend at least as strongly in the same direction, for its leaves resemble those of *S. Cinerea* as well as *S. Viminalis*, and *S. Cinerea* produces yellowish larvæ. And so with *S. Smithiana*, which resembles *S. Viminalis* and *S. Caprea* (which probably produces, yellow larvæ from its resemblance to a large *S. Cinerea*). But both these intermediate sallows produced, as a rule, intermediate varieties—that is to say, their effects were between *S. Viminalis* and *S. Cinerea* (or ? *S. Caprea*), just as their leaves combine the characters of these forms. And, further, in the intermediate varieties produced, the whitish form predominated, just as the characters of *S. Viminalis* predominate on the underside of the leaves. Hence the observations upon these two food-plants support my view as to the effect of *S. Viminalis*. In the case of *S. Triandra* I have some little experimental evidence, but I have never found a larva on this tree in the field. I feel strongly that its true tendency is, like that of *S. Rubra*, towards a protective (i.e., a yellowish) variety. This year I hope to breed a large number of larvæ from the egg upon this tree, and upon *S. Viminalis*. Omitting these two trees, the whole list is very favourable to the theory that the results are protective, and that the tendencies can be ascertained by looking at the undersides of the leaves.*

19. *The Complex Nature of the Influence of the Food-plant upon the Larvæ of S. Ocellatus.*

The simplest view of this matter would be to suppose that the colour of the leaf was itself the cause of the colour in the larva, showing through the transparent skin, and that the change of diet was therefore the direct cause of the change of colour. But it is quite clear that the influence cannot be of this simple kind, for the amount of substance which forms the underside of the leaf is a very small proportion of the whole substance that is eaten.

* I have to thank Mr. G. C. Druce for much kind help in the difficult task of naming the sallows.

It is, therefore, hardly conceivable that this small proportion can directly modify the whole larva into its own likeness;—that the under surface eaten by the larva should cause the latter to resemble the surface itself. And yet it is only this part of the food which is common to the various food-plants producing similar effects. In everything except whiteness and pubescence of the underside, the leaves of the apple must differ more widely from those of *S. Viminalis*, *Ferruginea*, and *Smithiana* than these do from *S. Rubra*. And yet the effects of the latter plant are very different from the three former species of sawfly, of which the tendencies incline towards those of apple. And further, the resulting larvæ are far better protected by such effects produced in common by apple and certain sawflies than if all the sawflies had tended in the same direction. Furthermore, the appearance of the undersides is quite as much due to texture as colour, for the pubescence produces a large part of the effect of dead whiteness. But the larva gains the same appearance by colour, for its hairs are microscopic rudiments (only discovered by myself last summer, 1884). Again, there is no reason for supposing that the chlorophyll from the leaves of the food-plants would yield solutions corresponding in colour with the larvæ; in fact, it is certain that this is not the case, for the colour of the upper sides of the leaves do not afford an indication of the effects on the larvæ, and yet the true colour of the leaf is far more distinct here than it is on the under surface.

These conclusions are also confirmed by considering the effects of the experiments. While the existence of an influence of the food-plant upon larval colouration is undeniably proved, the simple theory of its manner of action is entirely refuted, for such a theory does not allow for any powerful tendency on the part of the larva which may even counterbalance that of the food. Thus in the experiments the effects produced were not according to the power of the food-plant (as proved by observation), when the tendency was in the direction of yellowish, while the effects were very powerful when the tendency was in the other direction. And so also with the observations in the field, as was proved by the very different colours on the same tree, and the strongly yellowish variety upon apple. It cannot be argued that the irregularities observed are due to the effects of the food-plant varying in intensity upon larvæ of different constitutions. They can only be explained by the possession of an opposing force in the larva itself, which may not only exactly counterbalance the tendencies of the food, but may produce the result of the strongest opposite tendencies. The former explanation would account for all varieties from intermediate to whitish upon apple, and from intermediate to yellowish upon *S. Rubra*, but it fails to account for anything on the whitish side of intermediate upon *S. Rubra* or on

the yellowish side in the case of apple. Far less can it account for an almost typical yellow variety upon this latter plant.

It is clear from the experiments upon the captured larvæ that some effect may be produced in an intermediate variety by feeding it for some considerable time (such as during the last stage), upon a food-plant known to have strong tendencies. But this treatment does not affect a larva which possesses a well-marked colouration—that is, a larva with strong tendencies itself, and which coincide with those of the food-plant. Nevertheless the experiments with larvæ bred from the egg prove that the variety towards which the larva is strongly tending may be much modified by the food-plant given it during the whole of its life. When such a change has been produced and is manifest, if the food be appropriately shifted at the beginning of the fifth stage, some considerable effect may be produced in the larva, in the direction of the original tendency.

20. *A Suggested Explanation of the Larval Tendency towards Certain Colours Independently of the Food-plant.*

The most probable explanation of these larval tendencies—which differ so much in strength and direction, and which are independent of the food-plant though generally modifiable by it—is that phytophagic effects are hereditary, and thus stronger tendencies accumulate as the larvæ of successive generations feed upon the same food-plant or on those which produce the same effects. In this way all shades of colour may be occasionally found upon any tree, and the same irregularity may be produced by interbreeding between individuals with different tendencies. This explanation accounts for all the facts brought out by this year's experiment and observation, and it is also in harmony with previously recorded facts. Thus the unexpected results of the breeding experiments would be explained by supposing that during the previous generation (or perhaps generations), the larvæ had fed upon apple or some other food-plant tending to produce whitish varieties. The yellowish larva found upon apple must, according to this theory, have descended from a long line fed upon *S. Rubra*, or some plant with leaves having green glabrous undersides. In this case I believe that there must have been several generations accumulating yellowish tendencies by their food-plants, for the effects of apple are so very strong in the other direction, and yet so little change was produced. So also with regard to the two larvæ of different varieties upon the same tree, it may be supposed that there had been different tendencies due to the food-plants of past generations. In one of the cases quoted, there is some slight evidence that this reasoning is correct. Of the seven larvæ found upon *S. Babylonica*, one was yellowish, and the others intermediate.

According to this theory, the egg from which the former was hatched had been laid by a different female from that which produced the others. Such a conclusion is confirmed by the fact that the yellowish larva was decidedly older than the others and became adult some days earlier, the six intermediate larvæ keeping together throughout. I endeavoured to ascertain the past history of the parents of the larvæ with which I experimented, but Mr. Davis informed me that his larvæ had not been kept separate, and had been found on different food-plants.

It may be argued that there is probably great difference among individuals of the same brood in the power of transmitting these tendencies, and that this might explain some of the above-mentioned irregularities. But the breeding experiments, as far as they go, negative such a conclusion. There was very little difference between the larvæ of each separate lot, and considerable differences between the lots (in some cases). The influences of the food-plant seemed to be most rigidly regular in their effects on each division of the larvæ: in fact one might say that there were practically no individual differences in the tendency towards a whitish variety, although the tendency was very strong.

There is one argument against the conclusion that these effects are in any way due to the food-plants (but I do not see how it can stand against the breeding experiments):—the fact that the variably developed system of brownish-red spots have only been found on the yellowish-green larvæ up to the present time. (Mr. Meldola's instances point in this direction without exception, and so also with my own.) It is, however, quite possible that these spots may be ultimately found upon the other variety. If not, it would seem to imply that the yellowish-green form, often brightly ornamented with red spots, is more ancient than the other, and that reversion to it is caused by certain food-plants. Against this comes the fact that the red markings have all the excessive variability of a reversion character, while the yellowish-green ground colour can be produced with great regularity. Again, the spots occur upon larvæ with all shades of ground colour in *S. Populi*. Looking at all the facts I cannot doubt the cogency of the explanation offered above of the colouration being due to the food-plant, and of the accumulation of the influence during successive generations. It seems very probable that we have here an instance of a larva of one species dividing into two. The tendency is more of this kind than towards true dimorphism, for the two forms have different food-plants. If a long series of generations upon one plant produced a distaste for the other kinds, so that the larvæ wandered or died, it is then certain that natural selection would lead the females to lay their eggs on the appropriate foods, and the separation would be complete. Mere proximity of one kind of food and distance from

other kinds might produce some tendency in this direction. In gardens there are many chances in favour of eggs being laid for several generations on apple, and so with regard to the predominance of any one species of *Salix* over a particular area.

It would thus be possible for the two varieties to be rendered locally distinct, for the localisation of a food-plant would overcome both causes of variability—the liability to lay eggs on other plants with different tendencies, and the chance of interbreeding between the two varieties. If the two varieties could become stereotyped in this way (and it has been shown that *occasional* recurrence to other kinds of food on the whole produces no effect), there would result a species with specific differences in the larvæ, although the pupæ and imagos would remain identical. Instances very nearly of this kind are, of course, well known. The above would certainly be true of two isolated tracts in which food-plants of opposite tendencies existed to the exclusion of the other kinds. If the tracts were adjacent it would still be true of nearly all the larvæ in each of them.

It may be argued that the food-plants are so intermixed throughout this country that we should not expect to find the uniformity which has hitherto seemed to prevail. But it must be remembered that such uniformity is chiefly observed upon apple, and that the effects of this food-plant are so strong that it needs a very powerful tendency to overcome them. Furthermore, there is a certain amount of local separation between apple trees in gardens and the various species of willow by the banks of streams and in damp lanes and hedgerows. The species of *Smerinthus* are inactive and slow flyers in the perfect state; so there is every tendency towards interbreeding between those in gardens, and towards the eggs being laid upon the food-plant which is on the spot. These facts will account for the almost invariable occurrence of the whitish variety upon apple. The case is very different with the willows. The various kinds occur in close proximity, so that it is very common to find several so-called species of *Salix* in a single osier-bed. The tendency of willows as a whole is rather towards the yellowish variety than towards the whitish, but there is at all times the greatest facility for interbreeding, and for laying eggs on many species of food-plant, even in the case of the most sluggish insects. This explains the irregularity of the larvæ when found on willows, and the fact that the yellow variety is perhaps more commonly found upon the trees with an opposite tendency than the white variety upon trees tending towards the yellowish form. I may add that the garden at Reading in which I found the yellowish form upon apple, is on the outskirts of the town, and so situated that the eggs might easily be laid by a female of which the larva had fed upon willow, for these trees are common at no great

distance, and in fact there are examples of *S. Cinera* and *S. Babylonica* in the garden itself.

It seems therefore that the explanation offered above must be correct, and if so we have an instance of a character (larval colouration) that is shown in a very obvious manner to be the result of the interaction between the influences at work in an individual life and the inherited tendencies following from the influences that moulded the lives of ancestors. In this case, too, there is a fairly accurate test for the predominance of either element, in observing the resultant tint, and making allowance for the tendencies (already gauged) of the food-plant.

The case of *S. Ligustri* is also to be explained in a similar way, for here, too, we have the difficulty of conflicting experience (Mr. Davis' and my own). I have a considerable number of pupæ of *S. Ocellatus*, with careful notes of the colours and food-plants of their respective larvæ, so that I hope to be able to test the above theory very conclusively in the summer.

21. *The Essential Nature of the Changes in Colour Produced by Food-plants.*

It is first necessary to carefully describe the appearance of the varieties of *S. Ocellatus*, before explaining the underlying cause of the colour, by reference to larval or derived pigment.

The larva of *S. Ocellatus* is covered with minute white points (which are the tubercles at the bases of rudimentary hairs), conferring upon the skin a shagreened texture. The colour is much modified by these dots; and their arrangement, together with the spreading of white areas from their bases, and the coalescence of such areas, form nearly all the markings of the larva. The dots are very variably developed; at one time I thought that the whitish varieties must be caused by their relative predominance, obscuring and modifying the green ground-colour. Careful observation, however, convinced me that the difference is not due to this. The most extreme whitish varieties are almost white upon the back, above the horizon of the old subdorsal line. Below this the bluish-green tint predominates (the line of demarcation being very sharp), while the under surface is dark bluish-green. Careful examination of the white dorsal surface with a lens shows that the green is obscured (and almost obliterated) by a comparatively superficial accumulation of white masses, while the green when present seems to shine through from a lower level. On the under surface there is nothing to obstruct the effect of the green (since the shagreen dots are very minute, and the white masses are absent). The lateral surfaces offer intermediate effects produced by intermediate conditions. An

extreme yellowish variety, on the other hand, does not present the same marked line of demarcation at the subdorsal level; the whole larva is bright yellowish-green, the tint being more pronounced on the under-surface, but very bright and distinct over the whole larva. It is here also obvious that the main colour is deeply placed, and is to some extent modified by the shagreen dots and by subcuticular whitish masses, but these latter have not the same effect as in the other variety (and are probably developed to a much less extent).

A less extreme form of the yellowish variety differs in the dorsal tint being more completely obscured by white (the result being a yellowish-white in this region). The line of demarcation is not sharp. In an intermediate variety (such as that found upon *S. Ferruginea*), the upper surface resembles that of the whitish form, with the same sharp contrast at the subdorsal, but below this the tint is green, without any marked tendency towards yellowish or bluish, the under surface being of course the most deeply coloured. Such a variety is not at all uncommon, and I have described it as inclining towards the whitish side of intermediate. It is truly intermediate in the colour of the lateral and lower surfaces, inclining towards the whitish variety on the upper surface, and in the sharp demarcation at the subdorsal level. It is at once clear that the green tints, whether yellowish or bluish, are due to derived pigments, which have already been described as present in the blood of the pupa, and which have been proved to exist in the subcuticular tissues, as well as in the blood of the larva. The white masses are evidently due to a substance of true larval origin, and the effect of the food-plant upon the latter shows at once that its influence extends beyond the derived colours. The yellowish tint of the bright variety is due to predominant xanthophyll, as is proved by comparing the blood of pupæ of which the history is known. The greener variety must conversely be due to a larger proportion of chlorophyll, and the blue tinge may be caused by a slight change in its constitution; but this needs investigation in the larva, for although the pupal blood of yellow varieties is brighter in colour, that of the others does not retain the blue tinge. The difference is delicate, but distinct; and much must be lost in the redistribution accompanying pupation. It is likely that the blood of the larvæ is not very different, but that the chief distinctions are due to the subcuticular derived pigments, and these are largely destroyed in the changes preceding pupation. It is unfortunate that this inquiry did not suggest itself to me when I possessed the living larvæ in abundance.

The case of *S. Ligustri* is more distinct, for the differences in the green seem to be entirely retained in the pupal blood. I have now many times compared the blood of lilac and privet pupæ, and that

of the former was always greener, and gave the principal band of chlorophyll more distinctly when equal thicknesses were compared. It therefore appears that the blood and subcuticular pigment are of similar tints in this larva. In this case also the influence of the food-plant extends to the true larval pigments, for the purple stripes are much brighter in the privet forms.

There is very little doubt that the green and brown varieties of *Noctua* larvæ could be produced by a proper arrangement of surroundings, but as the experiment has not yet been tried, it must not at present be assumed that the food-plant could produce so great an effect as the removal of the derived pigments from the blood. Obviously the common experience that brown larvæ rest on the brown parts of the plant or on the earth is not a sufficient argument, as the habit may have followed the change of colour. But a change in the relative proportion of the derived pigments passed through the walls of the digestive tract into the blood seems to result from a more complex influence than that which would lead to the entire removal of pigment from the blood; and yet we have proved that the food-plant exerts the former influence.

22. *Summary; and Conclusion as to the Nature of the Influence of the Food-plant.*

It has been shown that the influence of the food-plant is not uniform, that it must act during a large proportion of the whole larval life in order to produce an effect, that effects of surface colouration due to consistence may be imitated in colour, and it has been rendered extremely probable that the effects accumulate during successive generations. It has been shown that the effects are partially due to pigment which is proper to the larva, and which has no immediate relation to the food-plant, while the changes produced in the derived pigments are even more complicated, and due to the predominance of one or other of the vegetal colouring matters in the tissues and blood, and before this in the materials which traverse the walls of the digestive tract (for the hypothesis that certain pigments are continuously destroyed after passing the digestive tract, until a certain colour is produced, is even more complicated).

Such effects are entirely inexplicable by the simple theory of phytophagic influence with which I was strongly prepossessed on approaching this inquiry. So impossible does it seem that the effect could be produced by the direct influence of the material which is eaten, that it would be wiser to abandon the term "phytophagic," at any rate in the sense of producing these changes. The term still holds good for the broad fact that pigments derived from the food-plant play a most important part in larval colouration, and further than

this, that such pigments afford the material which is moulded by some subtler influence into a likeness to a special part of the environment. Of the nature of this influence we know nothing at present, except that the whole investigation points towards a nervous circle whose efferent effects are seen in the regulation of the passage of pigments through the digestive tract into the blood, and finally the tissues, and in the colour of a certain amount of true larval pigment, while the afferent part of the circuit must originate in some surface capable of responding to delicate shades of difference in the colour of the part of the environment imitated. This interpretation is rendered unusually difficult by three facts: the gradual working of the process, often incomplete in a single life; the excessively complex and diverse result, and the special character of the stimulus (for it is only the part of the environment imitated which produces any effect, *e.g.*, the undersides of the leaves in the case of *S. Ocellatus*, and yet the environment, of course, includes both surfaces). In parallel cases (Amphibia, fish, &c.), as far as any parallelism exists, the stimulus acts upon the eye, and this may be true of larvæ also, but it is useless to speculate on the subject until further data have been acquired by experimental research.

Variations in the colour of the derived pigments in the blood occur apparently spontaneously and uselessly in the opaque *P. Bucephalus*, for there is a great difference in the yellowness (xanthophyll) of the blood of this pupa and in the amount of chlorophyll. Thus it is possible that the variation began in this way, and was afterwards rendered efficacious by co-ordination with the environment. But there are difficulties in the way of any suggestion founded upon observations on this species, for the existence of green blood seems to indicate a different method of colouration in former times, and if so, the variations in colour may be themselves remnants of a past susceptibility to the influence of environment.

EXPLANATION OF SPECTRUM CHART.

Spectrum 1.—The blood of the larva of *P. Meticulosa* (green variety) examined in a thickness of about .75 mm. by sunlight. The blood had been allowed to remain in an open capillary tube for about four days, and was then sealed up after it had evaporated to half its bulk.

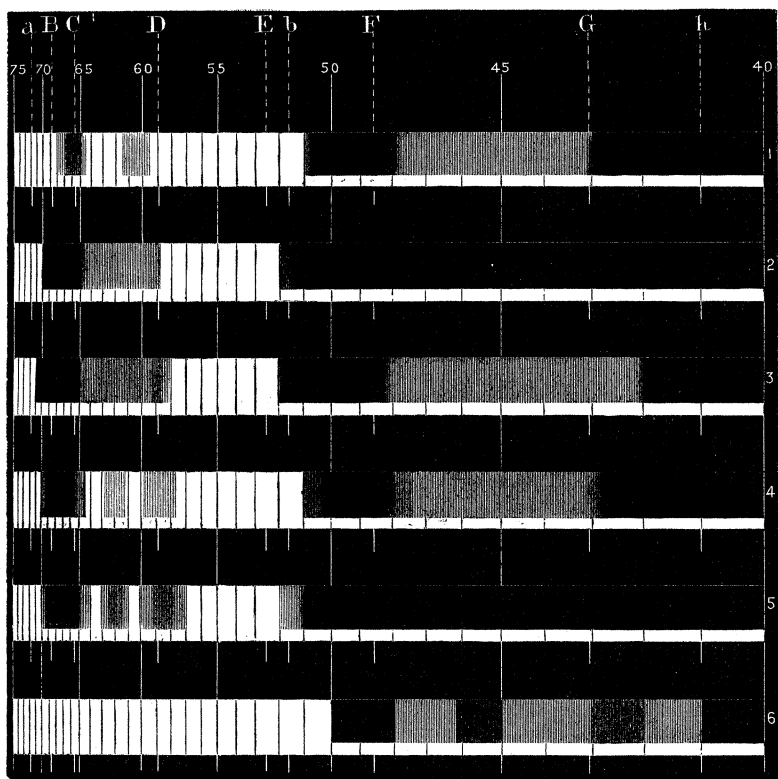
Spectrum 2.—The fresh and unaltered blood of the pupa of *S. Ligustri*, examined in a thickness of 35 mm. by sunlight.

Spectrum 3.—The fresh and unaltered blood of the pupa of *P. Bucephalus*, examined in a thickness of 23 mm. by sunlight.

Spectrum 4.—Two fresh calceolaria leaves, gently compressed and examined by sunlight.

Spectrum 5.—Five ditto ditto.

Spectrum 6.—The fresh and unaltered blood of the pupa of *S. Ligustri*, examined in a thickness of 3 mm. by illumination from the bright sky near the sun.



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